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ANNALS OF BOTANY

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AND OTHER BOTANISTS

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Researches on Plant Respiration

VI. The Respiration in Air and in Nitrogen of Thin Slices of Storage Tissues

BY

WALTER STILES

AND

K. W. DENT

With eight Figures in the Text

INTRODUCTION

IN the previous paper of this series (Choudhury, 1939) data were presented of the respiration in air, oxygen, nitrogen, and various mixtures of oxygen and nitrogen, of whole storage organs of a number of species. These included potato and artichoke tubers, and roots of carrot and red beet. The present paper deals with observations on the respiration of the same tissues in the form of thin slices.

The use of thin discs of storage tissue for the investigation of problems of permeability and salt absorption was developed some thirty years ago by Stiles and Jørgensen (1915), since when the employment of such material has become increasingly popular in researches on various aspects of the general physiology of plant cells. The chief advantage of storage tissue in this form as a subject for investigation lies in the fact that by employing as the experimental unit a number of discs of tissue taken from a stock of them prepared and treated as uniformly as possible, individual differences between replicate and comparable experiments, the bugbear of the plant physiologist, are greatly reduced as compared with those between whole organs. A further advantage these tissues have is their capacity for preserving their vitality over comparatively long periods. Thus one of us showed in 1927 that provided the supply of oxygen to, and removal of respiratory carbon dioxide from, the tissue is not unduly retarded, the latter can retain its vitality for several weeks. The chief disadvantage arises from the complexity of the tissue, including that induced by the wounding which is inherent in the preparation of the discs.

The observations of 1927 to which reference has just been made, clearly indicated the desirability of investigating the respiratory activity of discs of storage tissue under aerobic and anaerobic conditions, but owing to various circumstances, including the development of a method for measuring sufficiently small quantities of respiratory carbon dioxide (Stiles and Leach, 1931; Leach, 1932) and work on the respiration of germinating seeds and seedlings forming

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the subject of earlier papers in this series, experimental work on this subject was not begun until some six years later when a number of observations on the respiration of thin discs of potato tuber, red beetroot, and artichoke tuber under aerobic and anaerobic conditions were made in our laboratory by Miss Mary Colebourn. Since then a number of observations on the respiration of discs of storage tissue have been published, particularly by Turner (1938, 1938*a*, 1940) on carrot and by Bennet-Clark and Bexon (1943) on beetroot. Reference to the actual findings of these workers and of Miss Colebourn will be made in the course of this paper.

It is usual in experiments with discs of storage tissue for these to be washed in water for an arbitrary time before they are subjected to particular experimental conditions. Work by more than one observer has shown that the results then obtained may depend on the length of the washing period; that, in fact, the constitution of the discs is affected by the preliminary washing. The first question to be considered in this paper is therefore the effect of this preliminary washing on the respiration of the discs. This is followed by a consideration of the drift of respiration of tissue discs in air. The results obtained on the drifts of respiration in water and air suggest that these may be conditioned by the surface-volume relations of the discs. A glance at the values which have been obtained for the respiration rates of whole storage organs and of thin discs of the same tissues indicates that the respiration rate of a unit mass of the intact organ is generally very much less than that of unit mass of a thin slice of the tissue, and, falling in line with this, the respiration rate of unit mass of tissue falls with increase in disc thickness. We therefore next consider the bearing of the surface-volume relations of the tissue to respiration, a question which involves the fundamental problem of gaseous exchange and respiration of bulky tissues.

The last question to be discussed in this paper is the relationship of respiration of discs of storage tissue in nitrogen to their respiration in air. Although data on this relationship have been accumulating for many years, their importance in shedding light on the mechanism of respiration was first brought out by the well-known work of Blackman and Parija (1928) on apples. Information on the same subject with germinating seeds (Leach and Dent, 1934; Leach, 1936) and with whole storage organs (Choudhury, 1939) has already been presented in earlier papers in this series. Data with discs of one storage tissue, carrot, have been published by Marsh and Goddard (1939) and Turner (1940). Further data with this tissue as well as with red beetroot, artichoke, and potato are presented in this paper.

EXPERIMENTAL METHODS

Discs of tissue were prepared by cutting out cylinders from the respective roots and tubers by means of a cork borer and then cutting the cylinders into slices of the required thickness by means of a hand microtome with a screw possessing a pitch of 1 mm. The discs were cut with a diameter of 1.3 cm. In most of the work the discs were immediately transferred to aerated and

running tap-water in which they remained until removed immediately prior to the determination of their respiration.

For the measurement of the latter, two methods were used. For short-period experiments extending over only a few hours in which the discs were maintained in contact with water, the manometric method with the Barcroft type of manometer was employed. For the determination of carbon dioxide evolved as well as oxygen absorbed the so-called direct method was adopted in which two samples of tissue as equal as possible were allowed to respire over the same period of time in the right-hand flasks of two manometers, one with sodium hydroxide in the flasks, the other without. The constants for the instruments were obtained by calculation as described by Dixon (1943), and all precautions advocated by that author were adopted. Since the volume of tissue used was not negligible this was always taken into account in calculating the value of the constants, the water-content of the tissue being regarded as part of the aqueous phase in the flasks. In a few experiments in which the carbon dioxide output in absence of oxygen was measured flasks were used with side tubes provided with taps and a stream of nitrogen passed through the flasks for half an hour, after which all taps were closed. Any oxygen and carbon dioxide present in the nitrogen were removed by passing the gas through two flasks of an alkaline solution of pyrogallol. In the manometric experiments samples of tissue of from about 0.7 to 1.7 gm. were used and 3 ml. of water were always placed in each flask. All experiments of this kind were carried out at a thermostatically controlled temperature of 24° C.

For the measurement of respiration in air carbon dioxide output and oxygen absorption were measured by means of the self-registering katharometer and manometer (Leach, 1932) used in work described in previous papers in this series (Stiles and Leach, 1933; Leach and Dent, 1934; Leach, 1936). Measurements were continued for relatively long periods, sometimes for several days.

In these long-period experiments the usual quantity of tissue used in each experiment consisted of three discs 1 mm. thick and 1.3 cm. in diameter, although a few experiments were made with discs thinner and thicker than these. Special precautions were taken in the preparation of the discs to avoid bacterial contamination. After washing the tubers or roots thoroughly in tap-water they were next wiped with alcohol and then well washed with sterile distilled water. The cork borer, microtome, and razor used in cutting the discs were similarly sterilized, while the glass plant chamber was sterilized with concentrated nitric acid which was then completely washed away with sterile distilled water. The discs were held in the respiration chamber on a glass stand which was sterilized in the same way as the chamber. All experiments with the katharometer were carried out in water-baths thermostatically controlled at 25° C.

In all comparable experiments the discs were cut from one root or tuber. It was found that samples of discs as used in the manometric experiments did not differ in their respiration rates by more than 10 per cent. Two actual tests of the degree of agreement of such replicates may be quoted. In the

first the respiration rates of 3 samples each of 10 carrot discs, after 72 hours' washing in aerated running tap-water, were respectively 66.6, 68.6, and 63.0 μ l. per hour per gm. of fresh weight. In the second the respiration rates of 3 samples, each of 8 red beetroot discs, were respectively 98.7, 95.6, and 101.2 μ l. per hour per gm. fresh weight. On a dry-weight basis the results of the replicate experiments were in even closer agreement, those for carrots being 1257, 1276, and 1222 μ l. per hour per gm. of dry weight and for beetroot 1181, 1194, and 1203 μ l. per hour per gm. of dry weight. In the calculation of the respiratory quotient by the direct method, if the difference in the rates of respiration of the two samples of tissue used is as much as 10 per cent., the error introduced into the value of the quotient on this account is only of the order of 1 per cent.

Respiratory activity has been expressed in a variety of ways of which the most usual are mg. of carbon dioxide evolved per hour per gm. of fresh weight or dry weight and μ l. of oxygen absorbed per hour per gm. of fresh weight or dry weight. A reference to dry weight is not always more satisfactory than to fresh weight, and for the sake of comparison with the results of other investigators the experimental data recorded in this paper are frequently given in terms of both fresh and dry weights. As will appear later, in long-continued experiments with tissue discs in contact with water, both the fresh weight and dry weight change significantly with time, the former increasing considerably in the early stages, the latter falling throughout the whole course of the experiment. For the comparison of values obtained in one experiment the reference of respiratory activity to the original weight of the tissue would theoretically be sounder than reference either to fresh weight or dry weight, provided the discs of the whole sample were sufficiently uniform.

The primary data obtained by manometric methods are usually expressed as the volume of oxygen absorbed or carbon dioxide evolved, reduced to N.T.P., while with the katharometer the results are as readily expressed in one form as in the other. To convert μ l. of carbon dioxide at N.T.P. to mg., or vice versa, it is, of course, only necessary to multiply by a factor. Thus to convert μ l. of carbon dioxide at N.T.P. to mg. the factor is 0.001965.

A possible source of error in the determination of respiratory activity with the katharometer arises from the accumulation of carbon dioxide in the plant chamber. There is surprisingly little information about the effect of carbon dioxide on the rate of respiration of plant material, but Kidd (1915) found the respiration of germinating white mustard seeds and pea seeds was progressively decreased with increasing concentration of carbon dioxide, the effect on carbon dioxide output being greater than that on oxygen absorption, so that with increasing concentration of carbon dioxide in the environment there is also a progressive lowering of the respiratory quotient. As regards the effect on carbon dioxide production Kidd found that a concentration of 10 per cent. carbon dioxide in the atmosphere brought about a lowering of the rate of carbon dioxide output of approximately 10 to 40 per cent. according to the material and length of experiment. In the experiments with the katharometer

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the carbon-dioxide concentration in the plant chamber at the end of an experiment lasting 20 hours was sometimes as high as 2 per cent. but rarely higher, so that if Kidd's data for seeds are provisionally accepted as applicable to storage tissue, the respiration rate towards the end of an experimental period might be depressed on account of carbon dioxide accumulation in the plant chamber by from 2 to 8 per cent. An error of this magnitude would not affect in the least the conclusions to be drawn concerning the drift of respiration in storage tissue slices or the effect of changing from a normal atmosphere to one of pure nitrogen.

THE DRIFT OF RESPIRATION OF STORAGE TISSUE SLICES IN AERATED RUNNING TAP-WATER

It is a well-established fact that thin slices of storage tissue respire at a very much higher rate than intact organs. The rate is, however, not constant but exhibits a definite drift with time. As already mentioned, it is usual to wash the discs of tissue in water before actual measurements are made. Data on the drift of respiration of carrot and beetroot slices under these conditions have already been published by Turner (1940) and Bennet-Clark and Bexon (1943) respectively. The findings for the two tissues are very different. Turner's results with carrot slices 1 mm. thick, given a preliminary washing for 3.5 hours in running tap-water and then transferred to aerated distilled water at 22.5° C., indicate on the whole a high initial rate of respiration of about 30 mg. CO₂ per hour per 100 gm. fresh weight (= about 150 μ l. per hour per gm. fresh weight). For the first 50 hours the respiration exhibits wide variations with a general tendency to fall, after which the fall becomes more regular until after about 150 to 200 hours an almost constant, though still very slightly falling, rate of about 3 to 6 mg. CO₂ per hour per 100 gm. fresh weight (= 15 to 30 μ l. per hour per gm. fresh weight) is reached, a value about the same as that found by Turner for an intact carrot root and called by him the basal rate.

After a longer period of washing in tap-water the respiration rate appears to be lower than shortly after cutting and then drifts to the basal rate. Turner comes to the general conclusion that immediately after cutting the respiration is high and then drifts downwards with time until a value near that for the uncut root is reached after about 200 hours. The high initial value is attributed to stimulation caused by cutting, washing, and handling and perhaps also by intake of water or by ion uptake, while the comparatively high respiration rate of well-washed tissue when first transferred to the apparatus for measuring respiration may be attributable to contact stimulation and perhaps also to a temperature effect on the equilibration of substrate concentration related to transference of the tissue from washing-water at about 18° C. to the apparatus at 22.5° C. Experiments in which the respiration was determined manometrically were always above the basal rate and are of the same order as those obtained in the long-period experiments with the Pettenkofer method. The respiration rate as determined manometrically increased during the first 3 to

6 hours, though it probably fell subsequently, but Turner's conclusion is that the mean rate fell as the time of preliminary washing increased.

The conclusions of Bennet-Clark and Bexon on the drift of respiratory activity of discs of red beetroot stand in sharp contrast to those of Turner with carrot. The former workers, measuring the respiration rate of beetroot discs manometrically at a temperature of $24^{\circ}\text{C}.$, found that with continued washing in aerated tap-water at $12^{\circ}\text{C}.$ to $16^{\circ}\text{C}.$ the oxygen absorbed rose from a value of about $30\ \mu\text{l.}$ per hour per gm. of fresh weight shortly after cutting to about $130\ \mu\text{l.}$ after some 300 hours' washing, a value which did not alter materially for another 350 hours.

It should be noted that in all the experiments referred to above the discs are in water well supplied with oxygen. In Turner's long-period experiments with the Pettenkofer apparatus the discs were suspended in water through which a continuous current of air was passed, while in the manometric experiments, following the usual technique, the discs were contained in a small quantity (3 ml.) of water and were kept continuously shaken. Turner found, however, that the drift of respiration of discs in air was fundamentally the same as that of discs in water.

In the experiments to be described in this section of this paper the drift of aerobic respiration of tissue discs kept in aerating running tap-water at about $12^{\circ}\text{C}.$ was examined by removing samples at intervals and examining the respiratory activity manometrically. The usual technique was followed with the discs in 3 ml. of water kept continuously shaken. A few observations were also made with the katharometer on discs transferred from aerated running tap-water to air.

1. Beetroot

In the manometric experiments a stock of discs 1.3 cm. in diameter and 1 mm. in thickness was prepared from a single root and immediately transferred to running tap-water from which samples of 6 discs weighing about 0.75 gm. were removed at various times for the measurement of their respiration. Each experiment was continued for a period of from 2 to 6 hours. During this time the respiration generally showed a regular increase in rate, the average rate for the second 2 hours being often of the order of 10 per cent. higher than that for the first 2 hours. This behaviour is in complete agreement with Turner's experience with carrot. This rise in respiration rate of the tissues in the manometer flask is not to be confused with the drift of respiration of the tissue with time during prolonged sojourn in running aerated tap-water, as it occurs whether this latter drift is following an upward or downward course, and is, moreover, much more rapid.

The meaning of this rising respiration rate in manometric experiments is discussed by Turner. His conclusions, with which we agree, are in effect that the rise must be attributed either to the increase in temperature of the manometer flasks over that of the tap-water from which the discs are taken, or to the stimulation caused by handling the discs or by the continuous shaking

of the discs in the flasks, or to both these conditions. We are inclined to the view that the change to a higher temperature is the more probable condition inducing this rise, and that this is not a simple effect of temperature on respiration rate, since the tissue acquires the new temperature in a few minutes, but to an effect on the metabolites of the cell leading to a gradual increase in the concentration of the respirable substrate to a higher level, a change which might well take some time (cf. Turner, 1940, p. 278). Turner appears to regard contact stimulation as the more important factor, but, without denying the possible effects of this, it may be pointed out that the same rising course of respiration occurs whether the discs are removed from the running tap-water and well dried between filter-paper before transference to the manometer flask, or whether they are carefully removed by forceps from the tap-water and transferred directly into the manometer flasks without drying and so with a minimum of handling. It may be mentioned that no difference could be detected in these short-period experiments between the respiration of the discs in distilled water and in tap-water.

Since the respiration in the manometric experiments shows this rising rate, the mean respiration rate over the first 2 hours of each experiment with discs withdrawn from the stock at different times has been used to follow the drift of respiration of the tissues in running aerated tap-water. The results of a typical experiment are shown in Table I.

TABLE I

Drift of Respiration of Slices of Red Beetroot in Aerated Running Tap-water

Hours.	Oxygen absorption.		R.Q.	Dry wt.
	$\mu\text{l./hr./gm. fresh wt.}$	$\mu\text{l./hr./gm. dry wt.}$		Fresh wt.
18	56.4	714.5	1.01	0.07765
42	92.7	1243	1.05	0.07322
66	91.1	1315	1.04	0.07085
138	91.3	1476	1.01	0.06034
210	112.0	1814	1.05	0.06032
306	90.1	1625	1.00	0.05544
378	82.3	1647	0.99	0.05178
498	76.6	1443	0.90	0.05148
570	68.0	1350	0.885	0.04965
642	59.1	1164	0.85	0.04612

The respiration thus follows at first the same rising course that Bennet-Clark and Bexon found with this tissue and which they call 'ageing effects'. They regard these as due to less restricted gaseous exchange and to mechanical injury and wound stimuli resulting from cutting out the discs. While, as will be shown in a later section of this paper, the increase in respiratory activity of the discs after cutting over that of the whole organ from which they were cut is undoubtedly to be attributed to the former of these factors, and while the stimulus of wounding may also operate in the same way, one would at first sight at any rate expect them to be transitory effects. Since the respiratory activity may continue to increase for many days and then remain steady

at a constant rate, it would appear either that these consequences just mentioned of separating the discs of tissue from the intact organ induce metabolic changes leading to an increased concentration of respiratory substrate or of respiratory enzymes, as Bennet-Clark and Bexon suggest, or that these changes are dependent as well on the presence of an aqueous medium.

In our own experiments we have found that the maximum rate of respiration reached after several days' washing in aerated tap-water may be followed by a fall, as shown in Table I. Possible reasons for this are to be found in the continued diffusion out from the tissue of some cell constituent involved in the respiratory process or in exhaustion of the respiratory substrate. As regards the latter, the figures given in the last column of Table I are illuminating. After 642 hours the percentage of dry matter in the discs has fallen from 7.765 to 4.612. A small proportion of this decline is due to the prolonged absorption of water by the discs¹ and perhaps also to the exosmosis of solutes into the continually renewed washing-water, but undoubtedly by far the greater part of this loss is due to respiration. But since analyses show that soluble carbohydrates may comprise 60 to 70 per cent. of the total dry matter of the red beetroot, even after 642 hours there should still be a substantial reserve of respiratory substrate if all soluble carbohydrate is available for this. But inspection of the respiratory quotients indicates that this is probably not so. For the first 300 hours or so the respiratory quotients as measured were unity or slightly higher (1.00 to 1.05). After this they show a regular decline until after 642 hours the value is only 0.85. This fall in the value of the respiratory quotient has been observed in other tissues under conditions leading to starvation, and is generally attributed to the utilization of protein, giving a respiratory quotient of about 0.8, as the normal carbohydrate substrate becomes depleted.

2. *Mangold*

The effect of washing on the respiration of discs of mangold root with a thickness at cutting of 0.1 mm. was examined in the same way as in experiments with beetroot. As the data recorded in Table II show, the upward drift of respiration in mangold discs kept in running tap-water is similar to that in beetroot.

TABLE II

Drift of Respiration of Thin Slices of Mangold Root Tissue

Hours.	Oxygen absorption.		R.Q.	Dry wt.
	$\mu\text{l./hr./gm. fresh wt.}$	$\mu\text{l./hr./gm. dry wt.}$		Fresh wt.
22	31.2	431	1.04	0.07140
46	48.9	716	1.01	0.07136
166	102.4	1503	1.12	0.06246
334	127.7	2663	1.03	0.05040

¹ The average fresh weight of a disc increased from about 0.12 gm. after 18 hours in water to about 0.14 gm. after 138 hours and thereafter showed little change up to the 600th hour.

3. Carrot

Notable differences were observed in the respiratory activity of different carrot roots. In all the work described below the discs used in each experiment were obtained from a single root.

Discs 1.3 cm. in diameter and 0.1 mm. thick cut from the outer region of a root were kept in aerated running tap-water and samples abstracted from this stock for manometric measurement of their respiratory activity after various periods of washing up to 214 hours from cutting, by which time the stock was exhausted. As the numbers in Table III show, the respiratory activity increases throughout this time, and in this respect the tissue resembles beetroot. On the other hand, the results are in marked contrast with those of Turner, who, as we have already noted, observed a continuous decline in the respiratory activity of carrot discs, whether measurements were made by the Pettenkofer or manometric methods. In Table IV are given the results with discs of carrot cut from the central tissue. Although the respiration rates are lower than those shown in Table III, they show the same upward drift of the respiration rate which would appear to be followed ultimately by a fall as in beetroot.

TABLE III

Drift of Respiration of Thin Slices of Outer Carrot Root Tissue in Aerated Running Tap-water

Hours.	Oxygen absorption.		R.Q.	Dry wt.
	μ l./hr./gm. fresh wt.	μ l./hr./gm. dry wt.		Fresh wt.
22	128.1	1407	0.98	0.09145
46	135.5	1515	1.01	0.08864
118	138.4	1622	1.10	0.08557
214	156.6	1948	1.11	0.07999

TABLE IV

Drift of Respiration of Thin Slices of Inner Root Tissue in Aerated Running Tap-water

Expt. no.	Hours.	Oxygen absorption.		R.Q.	Dry wt.
		μ l./hr./gm. fresh wt.	μ l./hr./gm. dry wt.		Fresh wt.
H4 (b)	24	39.4	—	—	—
	46	64.1	—	—	—
	172	129.9	—	—	—
	410	113.0	—	—	—
	624	72.9	—	—	—
H15	49	45.05	759	1.04	0.05885
	144	55.0	934	1.09	0.0587
	456	79.6	1511	0.94	0.0503
	696	46.9	994	0.92	0.04825
H22	20	77.0	1196	—	0.06436
	68	115.4	1953	1.00	0.05690
	164	84.0	1497	0.91	0.05664

Although Turner's experiments with this tissue showed a short rise in respiration rate, this was over in about 10 hours, and he himself concludes

that apart from fluctuations over the first 50 hours the respiration rate follows a continually declining course. It is not possible at this stage to do more than suggest explanations for the difference in the behaviour of carrot tissue in Turner's work and in ours. The difference might be related to difference in the variety of carrot used, although this is unlikely. It is more probably related to the previous history of the tissue, that is, to its age and conditions of storage, and perhaps also to the composition of the tap-water in which the tissue slices were washed. Turner mentions that the Cambridge tap-water used in his experiments has a high ionic concentration whereas the reverse is the case with the tap-water of Birmingham. But he suggests that the high ionic concentration of the Cambridge tap-water, leading to ion uptake during the washing period, might be one factor inducing the high respiration rate observed at the beginning of an experiment. This rate in Turner's experiments was about 30 mg. carbon dioxide per hour per 100 gm. fresh weight, which corresponds to about 150 μ l. per hour per gm. fresh weight. This is much the same as the highest value observed in our experiments, but it is to be noted that considerable divergence occurred between samples from different roots. What is more important is that in none of the present experiments is the actual respiration drift of carrot similar to that observed by Turner; there is certainly not in general a continuous downward drift from an initially high value to a basal rate equal to that of an intact root, and no support is given to the view that the high respiratory activity of thin tissue slices is due to the stimuli of wounding and handling, and that as the response to these stimuli gradually dies away the respiration rate falls to the basal rate of the whole root. Turner himself points out that although for convenience the slowly falling rate reached in his experiments after 200 hours (about 25 μ l. per hour per gm. fresh weight) is termed the 'basal rate', it is not intended to imply that wound respiration and basal respiration are two distinct types of respiration. The results of the present researches lend no support to the conception of a 'basal' respiration rate in Turner's sense.

Indeed, the course of respiration of thin slices of carrot appears to be similar to that of beetroot except that higher respiratory quotients are observed. For the first 20 hours or so the respiratory quotient of discs in water in contact with air was found to be close to unity (cf. Tables III and IV), a result in complete agreement with those of Turner who obtained values of 0.98 and 1.00 for discs washed for 14 hours. The higher quotient with prolonged washing may be due to a certain amount of fermentation occurring concurrently with aerobic respiration or to the utilization of organic acid. According to Turner, unpublished results of El Gawadi have shown that aerated carrot discs produce alcohol in the quantities which agree with respiratory quotients of 1.01 to 1.13. The later fall in the quotient is most obviously explained as resulting from utilization of protein accompanying the exhaustion of the carbohydrate substrate.

THE DRIFT OF RESPIRATION OF STORAGE TISSUE SLICES IN AIR

In the experiments described in this section discs similar to those used for the experiments already described were transferred from aerated running tap-water to air at 25° C. in the katharometer chamber and records of the course of respiration obtained for various periods up to 5 days. In experiments which lasted for more than a day the air was renewed daily. The earlier experiments made in this laboratory by Miss Colebourn, to which reference has already been made, were of this kind, but larger discs of tissue, 2.2 cm. in diameter,

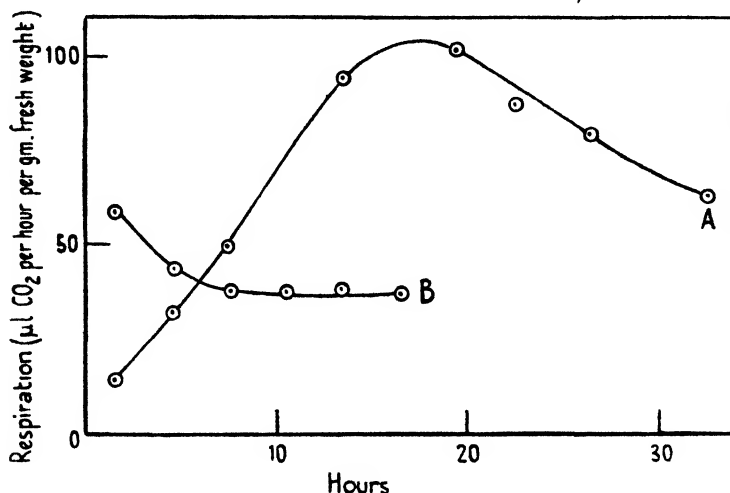


FIG. 1. Drift of respiration in air at 25° C. of slices of red beetroot 0.1 cm. thick. A, slices not subjected to preliminary washing; B, slices previously washed for 216 hours in aerated running tap-water at about 12° C.

were used and these were generally washed in several changes of distilled water. Where reference is made below to these experiments the pre-treatment is described.

1. Beetroot

Twenty-four experiments were made on the drift of respiration in air of discs of red beetroot after periods of washing in aerated tap-water varying from 0 to 216 hours. It has already been shown that the respiratory activity of these discs increases, within limits, as the washing period increases. Consequently we find that, in general, discs which have undergone no washing, or which have been washed for only a short period, begin respiring in air at a very much lower rate than those which have been washed for a longer period. But not only is the time of preliminary washing a factor in determining the initial value of the respiration rate in air, it also determines the subsequent course of respiration in air. After little or no preliminary washing the respiration rate rises rapidly, reaches a maximum after about a day, and then slowly falls. As the preliminary washing period is lengthened, the rise in respiration

rate is less, and with prolonged washing the respiration rate in air exhibits a falling course throughout.

The results of two sets of experiments illustrate this behaviour clearly. In the first, discs were cut from a beetroot freshly dug on September 5. Three discs 1.3 cm. in diameter and 1 mm. thick were transferred direct without previous washing to the katharometer plant chamber. The air was renewed 17, 25, and 41 hours after placing the discs in the respiration chamber. The course of respiration is shown in the second column of Table V. Other discs of the same stock were transferred to aerated running tap-water, where they remained for 216 hours, when three of them were transferred to air at 25° C. in the katharometer chamber. The course of their respiration is shown in the third column of Table V. The difference in the course of respiration resulting from prolonged washing is shown clearly in Fig. 1.

TABLE V

Drift of Respiration in Air of Discs of Beetroot not subjected to Preliminary Washing and after Washing for 216 Hours

Hours.	Respiration rate ($\mu\text{l./hr./gm. fresh wt.}$).	
	After no washing.	After washing 216 hours.
1.5	14.1	58.6
4.5	31.8	43.5
7.5	49.8	38.0
10.5	73.5	37.3
13.5	94.5	38.7
16.5	—	37.3
19.5	101.7	—
22.5	86.7	—
26.5	79.2	—
29.5	66.6	—
32.5	62.05	—
35.5	53.5	—
38.5	53.7	—
41.5	52.4	—
46.5	49.4	—
49.5	44.6	—
58.5	42.0	—
61.5	41.2	—

The discs used in the second set of experiments to be described were cut on November 19 from a beetroot taken from cool storage on that date. The course of respiration in air at 25° C. was followed with discs which had been washed for 0, 24, 72, and 168 hours respectively. The results are shown in Table VI.

From these experiments, which are representative of a large number, the following conclusions can be drawn. Firstly, the initial respiration rate in air at 25° C. increases with the length of the previous washing period in aerated running tap-water at 12°–16° C. at any rate with periods of preliminary washing up to 9 days. Secondly, the drift of respiration in air at 25° C. also depends on the length of the preliminary washing period. With no or little washing

there is at first a rapid rise in respiration rate for about a day, after which the rate remains approximately steady but with a general tendency to fall. As the washing period is made longer the rise in respiration rate becomes less, and if the washing period is sufficiently extended there may be no rise at all, but a slow decline in respiration rate with time.

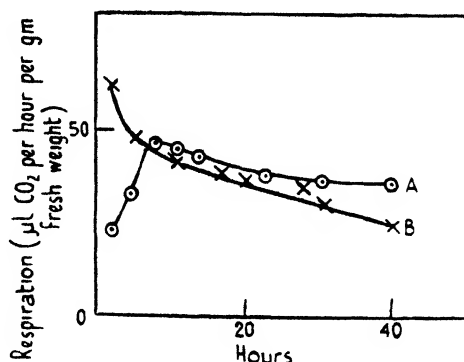


FIG. 2. Drift of respiration in air at 25° C. of slices of mangold root 0.1 cm. thick. A, slices previously washed in aerated running water for 1 hour; B, slices previously washed for 216 hours.

TABLE VI

Drift of Respiration in Air of Discs of Beetroot subjected to Different Periods of Washing in Aerated Running Tap-water

Time in hours.	Respiration rate (μl./hr./gm. fresh wt.).			
	After no washing.	After washing 24 hours.	After washing 72 hours.	After washing 168 hours.
1.5	16.1	35.2	35.5	49.0
4.5	17.6	41.9	37.4	52.7
7.5	28.6	51.3	41.0	49.3
10.5	38.1	54.5	44.7	54.0
13.5	48.7	59.0	44.4	50.5
16.5	51.1	57.85	48.2	52.8
19.5	73.9	55.9	45.1	53.2
22.5	74.1	—	—	—
23.5	—	54.9	—	51.2
26.5	—	50.4	44.4	45.0
29.5	—	48.6	42.3	41.2
32.5	—	48.6	39.9	40.2
35.5	—	48.1	40.2	37.2
38.5	—	47.2	39.5	39.9
41.5	—	47.1	37.0	37.1
44.5	—	46.5	—	36.4
47.5	—	—	—	34.9

2. Mangold

Eight experiments were made on the course of respiration of mangold discs in air. Two of these, in which the discs were washed in aerated running tap-water for 1 hour and 216 hours respectively, are summarized in Table VII and Fig. 2. They indicate that mangold behaves similarly to beetroot, the

respiration rate in air at 25° C. after a short period of washing starting at a low level and rising rapidly, whereas after prolonged washing the initial rate of respiration in air is high but falls regularly with time.

TABLE VII

Drift of Respiration in Air at 25° C. of Mangold Discs after 1 Hour and 216 Hours' Washing in Aerated Running Tap-water

Hours.	Respiration rate (μ l./hr./gm. fresh wt.).	
	After washing 1 hour.	After washing 216 hours.
2	22·6	61·8
5	32·7	48·6
8	46·5	38·2
11	45·2	41·3
14	42·1	38·4
17	41·7	38·2
20	41·3	36·8
23	38·7	—
25	—	37·5
28	35·7	35·0
31	36·5	30·0
34	35·5	32·55
37	33·7	26·3
40	35·1	24·7

3. Carrot

The course of respiration of thin slices of carrot in air at 25° C. appears to be similar to that of beetroot. Discs cut from the outer tissue of a root were washed in aerated running tap-water for 3 hours and then transferred to air at 25° C. in the respiration chamber of the katharometer. The air was renewed after 16 hours and again after 22 hours. The drift of respiratory activity under these conditions of the experiment are shown in Table VIII. The rise is characteristic of discs which have undergone washing for a comparatively short period.

TABLE VIII

Drift of Respiration in Air at 25° C. of Carrot Discs after 3 Hours' Washing in Aerated Running Tap-water

Hours.	Respiration rate	
	(μ l./hr./gm. fresh wt.).	
3	100·3	
6	116·3	
8·5	125·3	
20	131·7	
26	138·6	
29	142·0	

4. Potato

Two records of the drift of respiration of potato discs in air at 25° C. were made by Miss Colebourn. The discs, 2·2 cm. in diameter and 1 mm. thick, were washed in several changes of distilled water and transferred to the plant chamber attached to the katharometer about 5 hours after cutting. Four discs were used in each experiment and the air in the plant chamber was renewed

daily. As with beetroot previously exposed to a short period of washing, the respiration rate rose to a maximum after about 20 hours, after which, apart from fluctuations probably of no significance, the respiration rate showed little change for a further 80 hours. The constant rates reached in these two experiments were respectively about 916 and 661 μ l. of carbon dioxide per hour per gm. dry weight, corresponding to about 127.2 and 91.6 μ l. of carbon dioxide per gm. fresh weight. The respiratory quotient was found to be close to unity, the average values found in the two experiments being 0.98 and 0.97.

5. *Artichoke*

In a single experiment carried out by Miss Colebourn with artichoke discs, the drift of respiration in air was similar to that of beetroot. The discs were cut 2.2 cm. in diameter and 1 mm. thick and after about 5 hours' washing in several changes of distilled water were transferred to the plant chamber and the respiration followed for more than 60 hours. After an initial rise lasting only some 5 or 6 hours, respiratory activity fell somewhat and then remained constant for about 20 hours, after which it fell slowly.

From the observations recorded above we may draw the following general conclusions regarding the drift of respiration in thin slices of storage tissue. On first cutting out the discs from the storage organ their respiratory activity is low. This respiratory activity slowly increases in aerated running tap-water at temperatures from 10° to 14° C. to a much higher level at which it may remain constant for a time (Bennet-Clark and Bexon, 1943), but from which it slowly declines. If transferred to air at 25° C. immediately after cutting the rise in respiratory activity is much more rapid. When the discs are pre-treated with aerated running tap-water although, as just noted, the initial respiration rate in air at 25° C. is increased with increase in the period of washing up to a certain time, the subsequent rise of respiratory activity is lessened until with prolonged washing the respiratory activity in air may follow a continuously falling course.

The rise in respiratory activity of the discs after cutting is most obviously to be attributed to the changed environment of the tissue of the discs. In the next section of this paper reasons will be given for concluding that the tissues inside a bulky storage organ are under a very low oxygen and high carbon dioxide tension. Both these factors will condition a low respiration rate. The outer cells of a disc cut out from the storage organ are thus transferred to an atmosphere containing a very much higher oxygen pressure and a very low carbon dioxide pressure, and it is therefore to be expected that the respiratory activity of the tissues would undergo a sudden and considerable rise. The significant fact is that the rise is not sudden, but gradual, taking sometimes 200 hours or longer to reach a maximum in aerated running tap-water at 10°–14° C., the time taken to reach the maximum depending no doubt on the temperature of the washing water and probably also on the age of the organs in storage and perhaps on the size of the organ and the region

of the organ from which the disc had been cut. In air at 25° C. the development of respiratory activity is much more rapid, but the maximum rate of respiration may not be reached for 24 hours or more.

It would appear, then, that the changes in metabolic activity which result from the separation of thin discs from the mass of a bulky organ take time for their development. These changes might consist in a shift in the carbohydrate equilibrium in the cell resulting in the increase in concentration of the actual substrate, or in the activation of an enzyme or enzymes concerned in respiration. The subsequent slow fall in respiration rate after the peak is reached may be ascribed to gradual exhaustion of substrate or to the slow development of some depressant factor. Utilization of substrate from the most rapidly respiring cells at the surface of the discs at a rate appreciably higher than that at which carbohydrate diffuses from the more slowly respiring cells of the interior would account for the slow fall, but such an explanation is, in the present state of knowledge, only conjecture. In the experiments with the katharometer, in which the tissue is contained in a closed chamber, increase in carbon dioxide concentration might provide a depressant factor, but the fact that the respiratory activity showed no change in value on renewing the air indicates that this factor was not operative in conditioning the slowly falling respiration rate observed with discs respiring in air.

THE RELATION OF RESPIRATORY ACTIVITY TO SPECIFIC SURFACE

The separation of tissue in the form of thin discs from a bulky storage organ results then in a considerable increase in respiratory activity whether the tissue is kept in air or in aerated running tap-water, and this development of respiratory activity must be conditioned by the changed environment of the tissue, the most obvious factors being a rise in the oxygen, and decrease in the carbon dioxide, content of the environment. Some discussion of the conditions of oxygen and carbon dioxide concentration and of the respiratory activity inside a bulky organ such as a beetroot or potato is therefore desirable.

The view is probably not uncommonly held that the intercellular space systems of bulky organs provide the cells throughout the organ with a supply of oxygen in sufficiently high concentration to allow respiration to proceed aerobically at a rate not limited by oxygen concentration. But the rate of respiration per unit mass of the whole organ is very much less than that of a thin slice. Thus Choudhury (1939) found respiration rates at 25° C. of whole potatoes, carrots, beetroots, and artichokes of 2.3, 10.2, 8.0, and 6.4 μ l. carbon dioxide per hour per gm. of fresh weight respectively, whereas the respiration rates of discs of these tissues 0.1 mm. thick recorded in this paper reached up to 50, 14, 14, and 30 times these values.

The great difference in the respiratory activity of whole organs and tissue slices goes along with the difference in the proportion of surface to volume of the tissues. The increase in respiration rate with increase in specific surface is also shown by the observations of Steward, Wright, and Berry (1932) on the respiration rate of discs of potato of different thicknesses; with decrease

in the thickness of the disc from 0.6459 cm. to 0.0345 cm. the respiration rate of the discs in air at 23.2° C. increased from 0.0739 mg. to 0.3070 mg. carbon dioxide per gm. per hour. Likewise Boswell and Whiting (1938) recorded for potato an increase in respiration rate per unit mass with decrease in disc thickness and we have obtained similar results with discs of carrot, beetroot, and artichoke (cf. Table IX).

TABLE IX

Influence of Disc Thickness on Respiration Rate

Tissue.	Period of previous washing in hours.	Disc thickness in. cm.	Respiration rate.	
			μl./hr./gm. fresh wt.	μl./hr./gm. dry wt.
Carrot . .	21	0.0334	53.5	628
		0.050	53.3	637
		0.110	55.6	618
		0.233	45.9	500
Carrot . .	47	0.045	197.8	2444
		0.100	153.6	1965
		0.201	105.4	1357
Carrot . .	96	0.0445	92.6	1753
		0.100	60.1	1168
		0.200	42.55	856
Red beet . .	21.5	0.0258	59.14	934
		0.0463	64.6	975
		0.100	64.1	908
		0.210	45.7	664
Red beet . .	168	0.0625	115.0	1573
		0.125	92.3	1105
		0.210	79.1	908
Artichoke . .	48	0.0483	214.7	2020
		0.1215	185.8	1634
		0.220	126.2	1135

We have already noted Turner's view of the meaning of the very considerable difference in respiratory activity of a whole carrot and of a thin slice, namely, that the whole root respire at a basal rate and that the high respiratory activity of the thin slice is due to the stimulus of wounding and handling, and that as these effects die out the respiration of the slice falls to the basal rate. But it has already been shown that the respiratory activity of discs in aerated running tap-water may increase regularly for 200 or 300 hours. Such a long-continued rise is not characteristic of the response to a stimulus and cannot be attributed to the stimulus of wounding as such. On this view we might regard the long-continued rise of respiration in aerated running tap-water as the result of continued stimulation due to the movement of the water. However, the continued rise of respiration of discs in still air for 24 hours or more can hardly be explained on these lines. Even after 5 days under such conditions the respiration of thin discs may be many times that of the intact organ. While not denying the reality of the effect of mechanical stimulation

in increasing the rate of respiration, the response is relatively brief, as Audus' work (1935) with leaves has already shown and the following experiment with storage tissue discs indicates.

Three discs of red beet were cut from a freshly gathered root on July 10 and after washing in water for 5 minutes were transferred to the plant chamber of the katharometer at 25° C. The respiration rate rose rapidly and in the course of 12 hours rose from 61.4 to 105.2 μ l. carbon dioxide per hour per gm. of fresh weight. Air was renewed after 22 hours, and during the subsequent

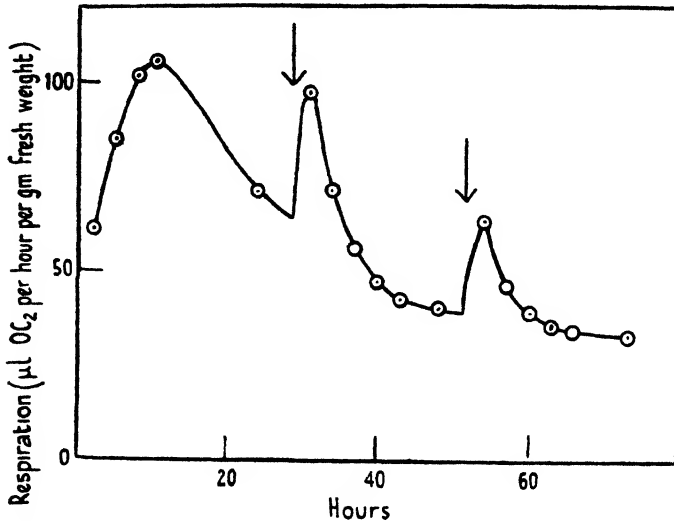


FIG. 3. The effect of mechanical stimulation, by bending, on the intensity of carbon dioxide output of thin slices of red beetroot. The times at which the stimulus was applied are indicated by the arrows.

6 hours the average respiration rate was about 66 μ l. per hour per gm. of fresh weight. The discs were then removed from the respiration chamber and each disc bent backwards and forwards 12 times and then replaced. The result was an increase in respiration rate of 50 per cent. during the next 3 hours, but the rate fell continuously and rapidly until after another 20 hours it had fallen to 40.7 μ l. per hour per gm. of fresh weight. The discs were then again removed from the respiration chamber and subjected to mechanical stimulation as before. Again there was a rise in respiration rate of about 50 per cent. followed by a fall which was rapid at first and then continued slowly until the experiment was ended after another 42 hours. The results of this experiment are shown graphically in Fig. 3 and indicate the very ephemeral effect of mechanical stimulation on the respiratory activity, the general drift of respiration not being affected.

The essential feature of Turner's point of view is that the stimulation of wounding when the discs are cut brings about a great increase in respiration rate over a basal respiration rate previously obtaining throughout the intact

organ and to which the high initial rate of the cut disc finally falls. Steward's view (1932), based on observations of the respiration rates of potato discs of different thicknesses, shows some resemblance to Turner's. Steward found his observed values could be explained by assuming a uniform low respiration rate throughout the disc, whatever the thickness, on which was superimposed a much higher respiration rate by a narrow superficial layer, this high rate being induced by the cells of the superficial layer coming into contact with oxygen.

Our own views differ in some respects from those of both Steward and Turner. The great difference in the respiratory activity of whole organs and tissue slices we consider to be mainly related to the difference in specific surface and the consequent difference in the oxygen and carbon dioxide concentrations inside the tissues.

The conditions in animal tissues have been considered by Warburg (1923) and Hill (1928). Considering a thin plate of tissue of infinite extent, as oxygen diffuses into this a gradient of oxygen concentration is set up and, assuming the actual consumption of oxygen by the cells is independent of the concentration, the oxygen concentration will be zero at a certain distance from the surface which is given by the expression $\sqrt{\left(2C\frac{D}{A}\right)}$, where C is the concentration of oxygen outside the tissue, D the coefficient of diffusion of oxygen in the tissue, and A the rate of respiration of the tissue. In a slice of tissue twice this thickness or less all the tissue is thus supposed to be respiring, and at the same rate; if the slice is thicker than this, only the outer layers $\left(\sqrt{2C\frac{D}{A}}\right)$ thick on each side are respiring; the inner region, to which oxygen does not penetrate, cannot respire. Assuming values for the diffusivity of oxygen and for respiration rate of 1.4×10^{-5} and 5×10^{-2} respectively, Warburg concluded that the limiting thickness of a slice of liver in which all the tissue was respiring was 2.1×10^{-2} cm. if the tissue was in air, and 4.7×10^{-2} cm. if it was in oxygen. Thus, up to a thickness of about 0.2 mm. the rate of respiration in air per unit mass of tissue would be independent of the thickness, but as the thickness of the slice is increased beyond this limiting value the rate of respiration per unit mass of the whole tissue will progressively fall with increasing thickness owing to the increasing proportion of non-respiring tissue in the middle region of the tissue.

Warburg's conclusions are not directly applicable to plant tissues because of (1) the presence of the intercellular space system of plants, (2) the incidence of anaerobic respiration (fermentation) in plant tissue when deprived of oxygen, and (3) the probability that the rate of respiration is not independent of oxygen concentration, at least when this falls below a certain value. The effect of the intercellular space system will be to increase the effective diffusivity of the tissue, while the incidence of anaerobic respiration will lead to a production of carbon dioxide in the inner region of the tissue where the oxygen concentration is below a certain value. This may, however, not

amount to much because, as carbon dioxide production continues, a gradient of carbon dioxide concentration will be set up in the tissue with the highest concentration in the middle of the tissue and the lowest at the surface, and this may suppress the production of more carbon dioxide. There is evidence of quite high concentrations of carbon dioxide inside bulky tissues. Boswell and Whiting (1940) found the average concentration of carbon dioxide in the internal atmosphere of potato tubers weighing about 70 gm. was 11.6 per cent. at 29° C., while Magness (1920) recorded still higher values, namely, 34.1 per cent. in potatoes at 22° C., and 28.6 per cent. in carrots at 24° C. Since these are average values for the whole cylinders cut out with a cork borer, it is clear that the values may be considerably higher in the middle of the tissues. The oxygen concentrations in the two tissues were respectively 5.7 and 5.2 per cent.

Our view of the respiratory conditions in storage organs is therefore as follows. Oxygen diffuses into the tissues from the outer air and a concentration gradient is set up. The considerations of Warburg and Hill, to which reference has already been made, indicate that, owing to the utilization of oxygen in respiration, the gradient is a steep one, so that at only a small fraction of a centimetre, probably less than a millimetre, from the surface the oxygen concentration is so low that it is limiting the rate of aerobic respiration. But in such conditions the production of carbon dioxide anaerobically is to be expected. It would seem, however, from measurements of the respiration and respiratory quotient of discs of storage tissue of different thicknesses that only a small proportion of the carbon dioxide is produced anaerobically, even in a thick disc. In an experiment with beetroot the respiratory quotients of discs cut at three thicknesses, 0.05, 0.10, and 0.40 cm., were found to be 0.968, 1.087, and 1.128 respectively, thus indicating a slight increase in the proportion of anaerobically produced carbon dioxide with increase in disc thickness. While we do not wish to lay much stress on this single experiment, it does indicate that the amount of anaerobically produced carbon dioxide in a disc even 0.4 cm. thick is only of the order of about one-tenth of the whole.

In the absence of experimental data we can only make a guess at the conditions of oxygen and carbon dioxide concentrations in the tissues, but if we assume that the effective diffusivity of oxygen in a storage tissue is 4 times that accepted by Warburg for liver, that the respiration rate in the surface layers of the storage tissue is 45 μ l. per hour per gm. fresh weight, and that this rate is constant with falling oxygen concentration down to the extinction point, a concentration of, say, 4 per cent., below which anaerobic respiration occurs, then, since the thickness of the superficial layer in which the respiration is entirely aerobic is given by the expression

$$t = \sqrt{2(C_0 - C_1) \frac{D}{A}},$$

where C_0 is the concentration of oxygen at the surface, C_1 the concentration

at the extinction point, D the diffusivity, and A the respiration rate, we have

$$t = \sqrt{\left(2(0.20 - 0.04) \frac{5.6 \times 10^{-5}}{4.5 \times 10^{-2}}\right) \text{ cm.}}$$

$$= 0.02 \text{ cm.}$$

This value would be about 40 per cent. higher if the effective diffusivity were twice or the respiration rate half that assumed, and 30 per cent. lower if the effective diffusivity were half or the respiration rate twice that assumed.

If we take the value of $t = 0.02$ cm., it means that in discs 0.05, 0.1, and 0.4 cm. thick respectively, anaerobic respiration is limited to middle regions 0.01, 0.06, and 0.36 cm. thick respectively as compared with the surface regions of 0.04 cm. (since there are two surfaces) in which the respiration is entirely aerobic. Hence the average respiration rate per unit volume in the middle of a disc 0.1 cm. thick where anaerobic respiration obtains is only about one-twelfth of that in the outer region, while in a disc 0.4 cm. thick the average respiration rate of the middle region is only about one-sixtieth of that of the superficial layer.

From this discussion it will appear that there is general agreement that the respiration of tissue slices, at any rate for some time after their preparation, is considerably higher than that of the whole organ from which they have been cut. There are, however, at least two possible explanations of the observed facts. On the view adopted by Turner, for example, the tissue of the intact organ respire at a basal rate, and the high rate of respiration of tissue slices is due to the stimulus of wounding and handling. As these effects disappear, the respiration of the slice slowly falls to the basal rate. It would appear that this view involves an assumption that oxygen tension is adequate to maintain a uniform rate of respiration throughout the whole organ, since the mean respiratory activity of this is as high as that of a thin slice after the effects of stimulation have died out. Steward's view is similar in so far as the whole of a tissue disc or cylinder of any thickness or length is assumed to respire at a low uniform rate. As a result of cutting and exposure of the cut surfaces to oxygen, the superficial layers respire at a very much higher rate, this respiration being in addition to the ground respiration of the whole disc. Although Steward speaks of 'surface respiration caused by wounding', he would apparently not regard it as a response to stimulation which gradually disappears.

The view put forward by the authors is different from those of both Turner and Steward, although we agree with the latter that in the tissue slice the greater part of the respiration is carried on by a thin superficial layer and that this is related to the higher oxygen concentration at the surface. We suggest that in bulky organs such as potatoes, beetroots, and carrots there are concentration gradients of oxygen and carbon dioxide and that the respiration rate at any place in the interior of the organ is conditioned by the concentrations of oxygen and carbon dioxide at that place. The available evidence suggests that the oxygen and carbon dioxide gradients are steep, and that

owing to this the respiration, including anaerobic respiration, falls rapidly to a negligible value, perhaps within a millimetre of the surface. On this view the difference in the rate of respiration of whole organs and pieces of tissue of different sizes is at once explained. The fact that on cutting out a tissue slice from a storage organ the respiration rate of the slice rises gradually from a low value to one many times the original suggests that oxygen is necessary to activate either substrate or enzyme system. Where a change to a higher temperature is involved this may also be a factor in bringing about an increase in active substrate, but it will be observed that the increase in respiratory activity of tissue discs gradually increases in aerated running tap-water without any rise in temperature occurring.

THE RESPIRATION OF THIN SLICES OF STORAGE TISSUE IN NITROGEN

The effect on the carbon dioxide output of plant material as a result of its transference from aerobic to anaerobic conditions varies considerably among different tissues. In germinating seeds the usual effect is a fall in carbon dioxide output on changing over from air to an atmosphere of nitrogen (Leach and Dent, 1934; Leach, 1936); in apples there may be a rise in carbon dioxide output on transference from air to nitrogen followed by a progressive fall (Blackman and Parija, 1928). Whole storage organs show diverse behaviour; with potatoes, beetroot, and artichoke there is a fall in carbon dioxide output as a result of replacement of air by nitrogen, the fall being much greater with potato than with the other two organs; with carrot, on the other hand, a rise in the carbon dioxide output was observed on transference of the roots from air to nitrogen (Choudhury, 1939). In all tissues, and particularly in bulky ones, there is a transitional period after substitution of nitrogen for air, during which the intensity of carbon dioxide output is not a true measure of respiratory activity but is partly conditioned by diffusion out from the tissues of carbon dioxide already in the intercellular spaces. To get the true rate of carbon dioxide production immediately after transference to nitrogen extrapolation from the values obtained after the completion of the transitional period is necessary.

The evolution of carbon dioxide by thin slices of carrot root in water and various solutions through which nitrogen was bubbled was examined by Turner (1940). He found that when the previous rate of aerobic respiration was high, transference from air to nitrogen brought about a rapid fall in carbon dioxide output, which gave place after about 3 hours to a slower and continuous rise for 12 hours or more to a maximum followed by a second fall. On the other hand, when the previous rate of aerobic respiration was low (Turner's 'basal rate') the first phase of falling respiration rate on transference to nitrogen did not occur, the rate rising from the beginning to the maximum reached after 14 hours or so in nitrogen.

In work described below the carbon dioxide evolution from thin slices of a number of storage tissues in nitrogen has been followed by means of the katharometer. The tissues examined include red beetroot, mangold, potato,

and artichoke. In all the experiments summarized below, discs of tissue, after a period of preliminary washing, were placed in the katharometer chamber and a record of the respiration in air obtained for some hours, after which a current of nitrogen was passed through the respiration chamber, the taps closed, and a record of the respiration in nitrogen obtained.

1. *Red beetroot*

A number of records of the drift of carbon dioxide output of discs of red beetroot were obtained about 12 years ago by Miss Colebourn. Four discs 2.2 cm. in diameter and 0.1 cm. thick weighing together about 1.5 gm. were used for each experiment. After cutting, the discs were washed for about 5 hours in several changes of distilled water. They were then transferred to the respiration chamber of the katharometer and a record obtained of their respiration in air over periods of about 17 to 24 hours. A stream of nitrogen was then passed through the respiration chamber, the taps of the latter closed, and a record of the carbon dioxide output obtained for a period of about 2 days. Air was then passed through the respiration chamber, the taps again closed, and a record of the subsequent respiration in air obtained. The results of these experiments (E. 13–19) are exhibited graphically in Fig. 4. Our more recent experiments were carried out on similar lines but differed in various details. In three experiments (J. 20, 26, and 27) three discs 1.3 cm. in diameter and 0.1 cm. thick were used. In experiment J. 20 the discs after cutting were washed for 74 hours in aerated running tap-water before transference to the respiration chamber and the measurement of the carbon dioxide output. The periods in air, nitrogen, and air were respectively 3, 21, and 15 hours. In experiments J. 26 and 27 the discs were rinsed in water for only about a minute before they were placed in the respiration vessel, and the periods in air, nitrogen, and air were respectively 26, 24, and 41 hours in experiment J. 26, and 18, 24, and 23 hours in experiment J. 27. In the remaining two experiments (J. 33 and 34) three discs 0.2 cm. thick were used. After a rapid rinsing in water the discs used in experiment J. 33 were transferred to the respiration chamber of the katharometer, the successive periods in air, nitrogen, and air being 26, 21, and 23 hours. In experiment J. 34 the discs, after a rapid rinsing in water, were transferred to the respiration chamber through which a stream of nitrogen was immediately passed. After complete replacement of air by nitrogen a record of carbon dioxide output was taken over a period of 22 hours. The nitrogen was then replaced by air and a record of the carbon dioxide output obtained. After a further 24 hours air was replaced by nitrogen and this again by air after another 24 hours. The drifts of respiration observed in these various experiments are shown graphically in Fig. 5.

It will be seen that the respiration drifts are in general the same whatever the disc thickness and whatever the length of the preliminary washing period. There is first the rise in respiration rate in air to a maximum followed by a fall which has already been noted. On transference to nitrogen the output of carbon dioxide falls continuously for 15 hours, after which the rate remains

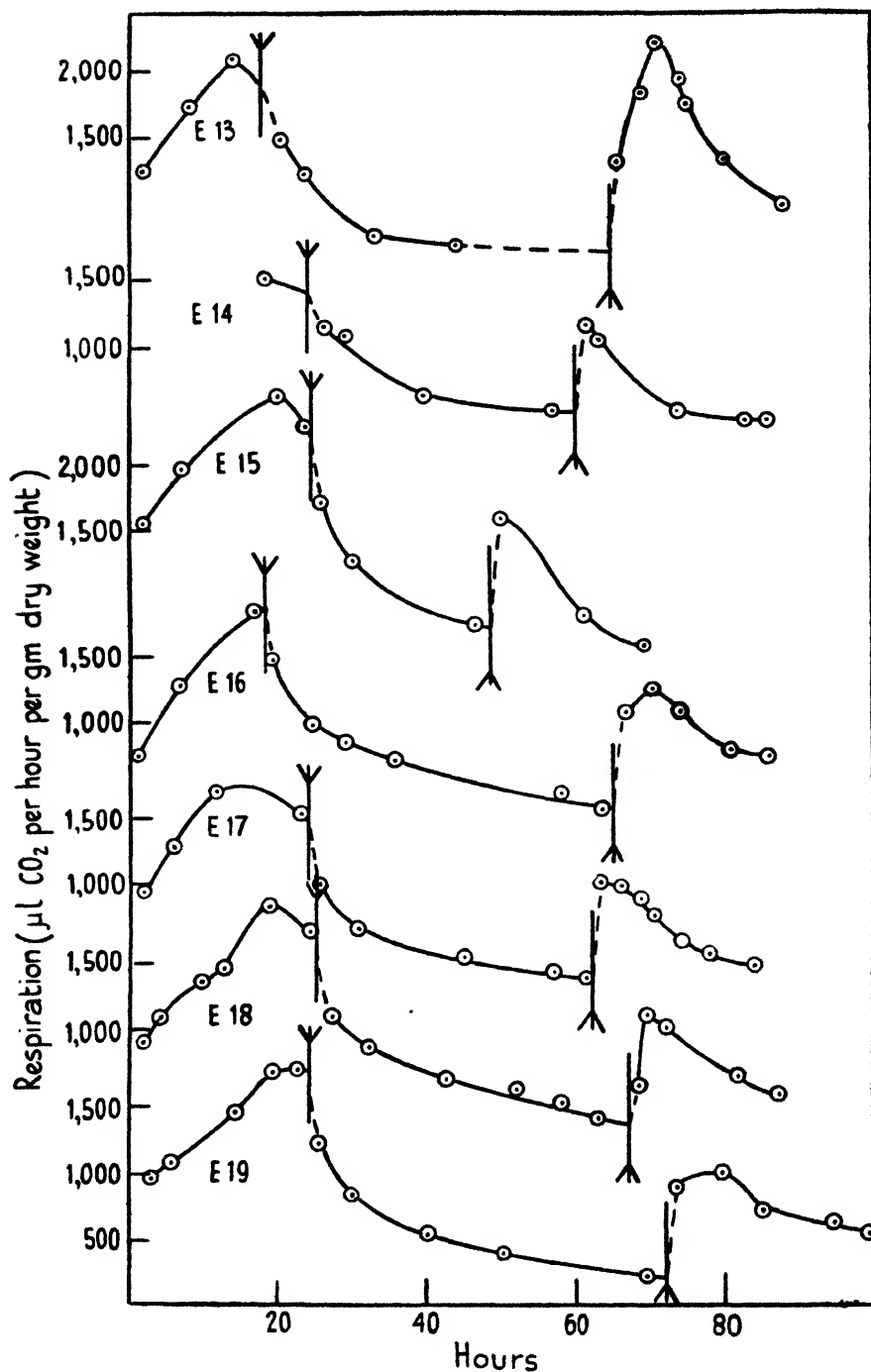


FIG. 4. The drift of respiration in air and in nitrogen at 25° C. of slices of red beetroot 0.1 cm. thick. The results of seven experiments (E. 13-19) are shown, in all of which the slices first respired in air, then in nitrogen, and then again in air. The times at which air was replaced by nitrogen are shown by the arrows ↓ and the times of subsequent replacement of nitrogen by air are shown by the arrows ↑.

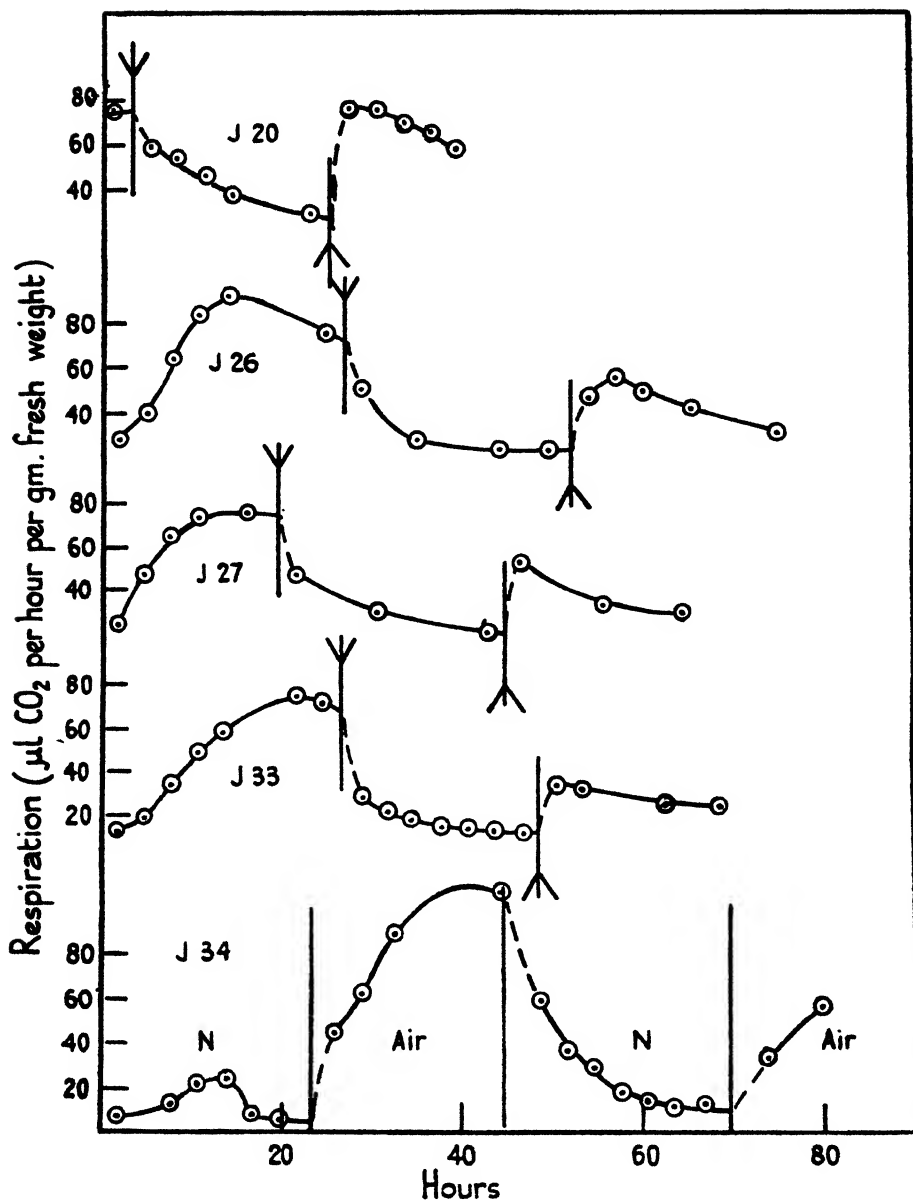


FIG. 5. The drift of respiration in air and in nitrogen at 25° C. of thin slices of red beet-root. The results of five experiments are shown, in four of which (J. 20, 26, 27, 33) the slices first respired in air. In these experiments the times at which air was replaced by nitrogen are shown by the arrows ↓, and the times of subsequent replacement of nitrogen by air are shown by the arrows ↑. In the fifth experiment (J. 34) the slices first respired in nitrogen, then in air, next again in nitrogen, and finally in air.

approximately constant, although with a slight tendency to fall, for at least another 25 hours. On replacement of nitrogen by air there is a considerable rise in carbon dioxide output to a value well above that of the air line, followed by a fall to the air-line level. The course of respiration of thin slices of beetroot in air, nitrogen, and air again is thus closely similar to that of whole roots (cf. Choudhury, 1939, Fig. 11).

2. Mangold

The results of two experiments with mangold are shown graphically in Fig. 6. In each three discs 1.3 cm. in diameter and 0.1 cm. thick were used.

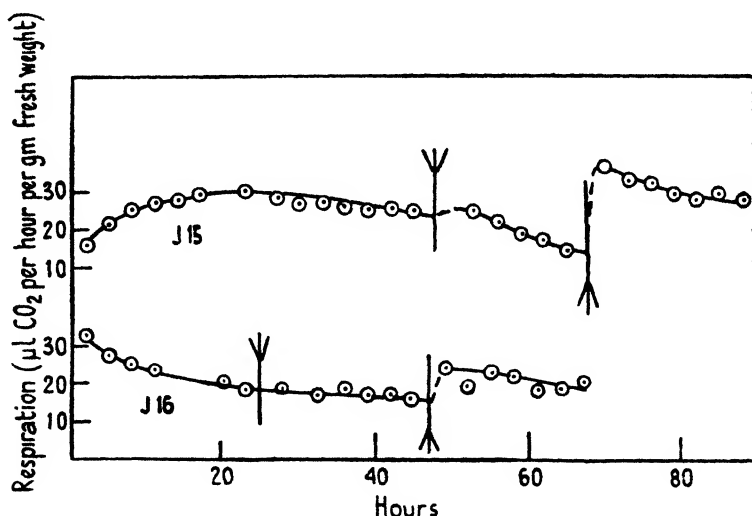


FIG. 6. The drift of respiration in air and in nitrogen at 25° C. of slices of mangold root 0.1 cm. thick. The times of changing the gaseous environment of the tissue are indicated as in Fig. 4.

In one (J. 15) the discs were washed for 75 minutes in aerated running tap-water before transference to the respiration chamber, in the other (J. 16) the discs, which were from the same batch as those used for experiment J. 15, were subjected to a preliminary washing of 216 hours. The drift of respiration in the two experiments was similar to that of beetroot except that instead of a rapid fall in the rate of carbon dioxide output on transference to nitrogen there was initially a slight rise. After this, however, the respiration rate fell slowly. On replacement of nitrogen with air there was again the rise in the rate of carbon dioxide output to a value above the level of the air line.

The difference in behaviour of beetroot and mangold exhibited in our experiments may be related to the age of the tissue in storage. The experiments with beetroot were conducted in the summer and autumn on tissue from roots which had undergone a relatively short time in storage, whereas the experiments with mangold were made in June on tissue taken from roots of the previous season and which had thus been in storage for many months.

Probably on account of their respective previous histories, the respiratory activity of the beet was much higher than that of the mangold. Now Turner (1940) found for carrot that transference of tissue slices to nitrogen from air when the respiratory activity was high resulted in an immediate rapid fall in the rate of carbon dioxide output, whereas when the respiratory activity was low there might instead be an immediate slight rise in the rate of carbon dioxide evolution.

3. Potato

In whole potatoes Choudhury found that on transference to nitrogen the rate of carbon dioxide output fell continuously for a long time, 40 to 50 hours,

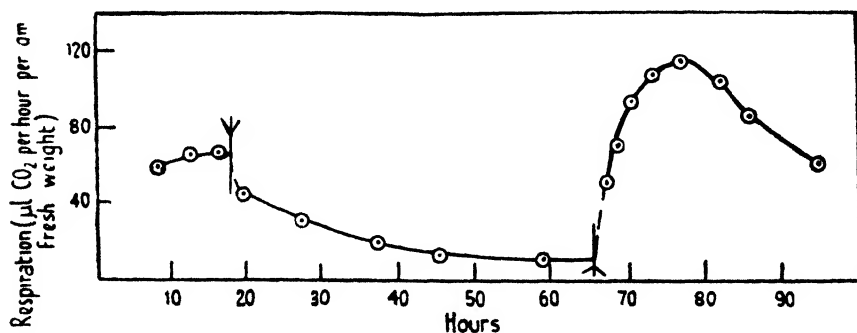


FIG. 7. The drift of respiration in air and in nitrogen at 25° C. of slices of potato tuber 0.1 cm. thick. The times of changing the gaseous environment of the tissue are indicated as in Fig. 4.

before reaching an approximately low and constant rate. In an experiment carried out by Miss Colebourn with discs of potato tuber 2.2 cm. in diameter and 0.1 cm. thick a similar result was obtained (see Fig. 7). Here also return to air brought about a considerable and temporary rise in respiratory activity to a value well above the air line.

4. Artichoke

A number of experiments (E. 23, 25-8, 30) were carried out by Miss Colebourn with discs of artichoke 2.2 cm. in diameter and 0.1 cm. thick. Her results are reproduced graphically in Fig. 8. Generally, immediately on transference to nitrogen from air there was little change in the rate of carbon dioxide evolution, although for about 14 hours there was a slow and continuous fall to an approximately constant lower level of carbon dioxide output. Transference back to air brought about a slight, though not very significant, rise in the rate of carbon dioxide evolution.

The points of interest in the results of the experiments described in the present section of this paper, and which call for discussion, are (1) the relation of the initial rate of carbon dioxide production in nitrogen to the final rate of carbon dioxide production in air, (2) the actual drift of carbon dioxide

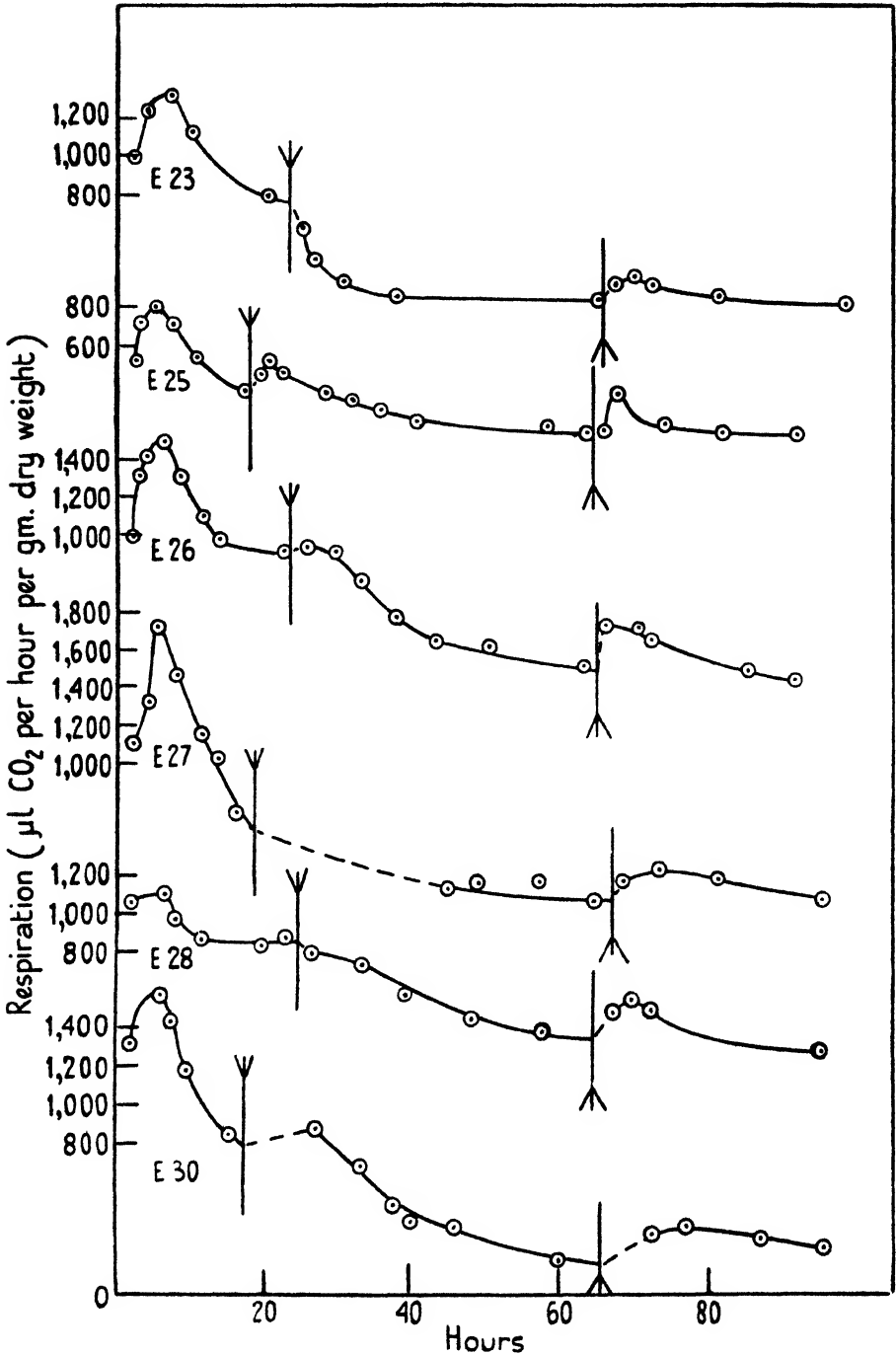
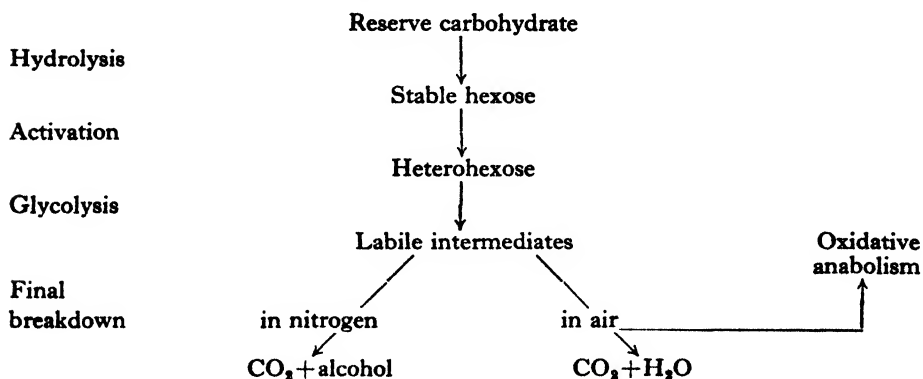


FIG. 8. The drift of respiration in air and in nitrogen at 25°C . of slices of artichoke 0.1 cm. thick. The times of changing the gaseous environment of the tissue are indicated as in Fig. 4.

output in nitrogen, and (3) the course of carbon dioxide output after replacement of nitrogen by air.

Before discussing these questions we may recall that F. F. Blackman (1928) has considered very fully the implications of similar data obtained for apples by Parija (1928). Blackman's well-known scheme for the respiration of apples we may accept as a basis for the discussion of our own results since it affords a satisfactory explanation of experimentally obtained data. According to this scheme we recognize in the breakdown of carbohydrate in the catabolic processes with which we are concerned a chain of reactions, namely, hydrolysis, activation, glycolysis, and respiration in a narrow sense, the first three of which are the same whether the tissue is in air or nitrogen, while the fourth, which we call final breakdown, takes a different course according to whether conditions are aerobic or anaerobic. Blackman's scheme is summarized thus:



We may now turn to the first of the three questions noted above, namely, the relation of the initial rate of carbon dioxide production in nitrogen (INR) to the final rate of carbon dioxide production in air (FOR). Blackman gave reasons for supposing that INR is a measure of the rate of glycolysis in air immediately preceding the change to nitrogen. Now for reasons that are so well known that they do not need repetition here, if INR is more than one-third of FOR there is, on Blackman's scheme, evidence of oxidative anabolism. The curves presented in Figs. 4-8 show clearly that in every instance the value of INR obtained by extrapolation is greater than one-third of FOR. While exact evaluation of INR must necessarily be somewhat approximate, it may be reasonably concluded that in the experiments with beetroot the ratio INR/FOR varied from about 0.5 (J. 33) to unity (J. 26), in mangold the ratios in the two experiments recorded were about unity and 1.2, while in artichoke the values for the ratio appeared to approximate to unity or somewhat higher. We may note that Turner (1940) found with carrot root that the ratio of INR/FOR varied considerably between, in fact, about 0.47 and 1.4, a range similar to that found in the tissues we have examined. Turner found the ratio tended to be higher the lower the value of FOR, and it is possible that the ratio varies in the same way with the storage tissues we have

examined; our results are not inconsistent with this finding, but the range of values of FOR found in our experiments was hardly wide enough to justify a definite pronouncement on this point. We may, however, conclude that if Blackman's hypothesis is accepted as applicable to the storage tissues we have examined, a considerable amount of oxidative anabolism occurs in all of them, this process accounting for from about one-third to three-quarters of the whole of the carbon involved in glycolysis in air.

It is, of course, possible to account for values of INR/FOR in excess of one-third in other ways. These have been considered by Turner (1937). There is the view of Lundsgaard that sugar breakdown follows a completely different course in presence and absence of oxygen; that in fact aerobic respiration and anaerobic respiration (fermentation) are unconnected processes. It might be urged that the lack of constancy in the ratio INR/FOR supports this view, but on the other hand the Lundsgaard hypothesis would not explain the general presence of fermentation enzymes in cells, one of the main reasons for the wide acceptance of Pfeffer's theory of the connexion between aerobic and anaerobic respiration. If we accept this connexion the only alternative to the Blackman hypothesis is the supposition that glycolysis, or the production of the substrate for glycolysis, is retarded by oxygen. Blackman rejected the possibility of oxygen reducing glycolysis, since, as he wrote, 'it is well known that oxygen has no direct effect upon the zymatic glycolysis of sugar in yeast'. Subsequently, however, Lipmann (1933) obtained data showing that although glycolysis in muscle extract is the same in air and nitrogen, if quinone or iodine is added in low concentration to the extract the output of carbon dioxide in air is considerably lowered. When an oxidation-reduction system such as hydroquinone-quinone or dichlorophenol-indophenol is added to the muscle extract there is a considerable reduction in carbon dioxide output in air but none or almost none in nitrogen. Lipmann concluded that an enzyme involved in glycolysis is oxidized under these conditions and rendered inactive, so that in air, provided the necessary oxidation-reduction system is present, there will result a lowering of the rate of glycolysis.

We should note that in this work Lipmann was concerned with that type of glycolysis characterized by the production of lactic acid, and it is therefore doubtful what bearing his results have on the problems of plant respiration. However, later (1934) he found a similar reduction in the production of carbon dioxide by fermenting yeast juice in oxygen if an oxidation-reduction system, namely, naphthol-sulphonate-indophenol, was present.

With regard to Lipmann's finding we may note that Marsh and Goddard (1939) found that the output of carbon dioxide from carrot-root slices in nitrogen was unaffected by cyanide and carbon monoxide, and only slightly affected by sodium azide, whereas all these substances bring about a considerable degree of inhibition of aerobic respiration. On the other hand, they induce a considerable amount of fermentation in presence of oxygen, as respiratory quotients between 3.0 and 4.7 were observed with carrot slices in aerobic conditions in presence of these respiratory poisons. Marsh and

Goddard point out that, if Lipmann's view is correct, it follows that these respiratory poisons must not only inhibit respiratory enzymes, but must also inhibit the action of the oxidizing system which Lipmann supposes inhibits glycolysis.

It is generally supposed that in fermentation during the course of glycolysis pyruvic acid is produced which as a result of the action of carboxylase breaks down to acetaldehyde and carbon dioxide. The acetaldehyde does not accumulate but is thought to react with phosphoglyceric aldehyde produced earlier in glycolysis to give ethyl alcohol and phosphoglyceric acid, the former accumulating while the latter breaks down to phosphoric acid and pyruvic acid. The phosphoric acid again takes part in preglycolytic reactions, while more acetaldehyde and carbon dioxide are produced from the pyruvic acid by the action of carboxylase.

Now it is well known that if a sulphite is added to the system, aldehyde sulphite is formed and the reaction of the aldehyde with phosphoglyceric aldehyde is thereby eliminated with the result that alcohol is no longer formed, the end products being in fact carbon dioxide, aldehyde, and glycerol. The effect of adding sulphite is thus to alter the course of the final breakdown.

It seems to us that the results of Marsh and Goddard suggest that we can look on the effect of oxygen on the course of the final breakdown in a similar way. In absence of oxygen the breakdown of carbohydrate follows the same course as in fermentation. If oxygen and a respiratory enzyme system are present the course of the final breakdown is altered, aldehyde or some other intermediate forming the substrate for the respiratory enzyme system. Just as when sulphite is removed the normal course of fermentation will be resumed, so when the respiratory enzyme system is inhibited fermentation occurs as Marsh and Goddard have shown.

Such a view does not involve as a necessity the inhibition of carboxylase, or any other enzymes involved in glycolysis, by the oxidizing system hypothesized by Lipmann, but it does not rule out the possibility of such an inhibition. If Lipmann's results are accepted as applicable to plant tissues in general they could be interpreted as indicating no more than what we already knew, namely, that the process of fermentation in plant cells is inhibited in the presence of oxygen and an oxidation system such as is involved in the normal respiratory mechanism. Even in Lipmann's experiments there is no need to assume that the inhibitory effect is on the glycolytic enzymes concerned in the breaking down of the heterohexoses; it might be an effect on the fermentation enzymes involved in the final breakdown of the labile intermediates to carbon dioxide and alcohol. Lipmann's results with muscle and yeast, if indeed it is justifiable to apply them to plant tissues generally, would then not be contradictory to Blackman's conclusion that glycolysis itself is not affected by oxygen. Blackman concluded that the rate of glycolysis is actually higher in air than in nitrogen as a result of the increased concentration of the substrate for glycolysis which results from the favourable effect of oxygen on pre-glycolytic stages in the chain of reactions involved in respiration.

From work with brain tissue Dixon and Holmes (1935) suggested that oxygen affects cell permeability so as to limit the rate at which glucose can reach the cell enzymes. In absence of oxygen the permeability of the cell is increased so that the enzymes become saturated with the substrate and the rate of glycolysis will become maximum. In the absence of any precise data with plant tissue we can at present only bear in mind this hypothesis for accounting for the Pasteur effect.

We come now to the second question for our consideration, namely, the drift of carbon dioxide output in nitrogen. The outstanding feature of this is the steady and long-continued decline in respiratory activity (fermentation rate). This is obviously not a diffusion effect, for in thin slices a diffusion lag would be of short duration; in carrot slices 0.1 cm. thick Turner concluded that the diffusion lag period was 2 hours, and it is reasonable to suppose that in beetroot the period would be approximately the same as in carrot. The prolonged fall in the rate of respiration in nitrogen may therefore be ascribed to a fall in nitrogen in the rate of glycolysis, which in turn might be due either to a partial inactivation of fermentation enzymes or to a reduction in the rate of the production of active substrate from reserve carbohydrate. The condition appears to be similar to that in apples, where Blackman concluded that in nitrogen there is a gradual decline in the rate of glycolysis, due, as we have already indicated, to the effect of oxygen in determining the activity of enzymes concerned in the preglycolytic stage so that in nitrogen the concentration of the substrate for glycolysis progressively decreases.

Another possible factor in bringing about the progressive decline in the rate of carbon dioxide output in nitrogen is the accumulation of alcohol or some other end product of fermentation in the cells which might result in an inhibition of fermentation enzymes. If such a factor does indeed operate, the action of the alcohol must be rapidly reversible, for after transference to air the drift of respiration soon resumes that which would have obtained had the tissue remained in air all the time (the air line).

The last matter for our consideration is this one of the course of respiration after replacing nitrogen by air. Very generally this re-transference, as with whole storage organs (Choudhury, 1939), is a sudden rise in carbon dioxide output to a value well above that of the air line followed by a fall to the air line level. This sudden outburst of carbon dioxide has been observed more than once in the past and has generally been attributed to the oxidation of material which has accumulated during the period of anaerobiosis. This accumulated product is not very likely to be alcohol, but whether it is some final product formed along with alcohol, or whether it is some intermediate in the formation of the final product, there is not at present evidence to decide.

SUMMARY

The respiratory activity of thin slices of storage tissues, beetroot, mangold root, and carrot root, continuously washed in aerated, running tap-water at about 12° C., rises slowly to a maximum which is not reached until several

days after the slices are cut out of the intact organ. This slow rise is followed by a slow and long-continued fall in respiratory activity. The phase of rising respiratory activity may correspond with the slow development of active respiratory substrate or of respiratory enzymes as a result of the access of oxygen to tissue previously enclosed in a bulky organ. The subsequent fall in respiratory activity appears to be related to a progressive lessening in the concentration of substrate. It is accompanied by a fall in the value of the respiratory quotient from about unity to 0.9 or less, suggesting the partial utilization of protein and the oncoming of starvation.

The respiratory activity of thin slices of storage tissue maintained in air at 25° C. and subjected to little previous washing rises rapidly to a maximum, reached usually in about a day, and then falls slowly. As the period of preliminary washing in aerated tap-water is increased, the initial rate of respiration in air increases and the increase to a maximum is less pronounced. With a preliminary washing period of 216 hours the respiration rate in air is initially high, but falls continuously.

A consideration of the relationship of respiratory activity to specific surface leads to the conclusion that in a bulky organ such as a beetroot or potato tuber the rate of respiration at any place is conditioned by the concentrations of oxygen and carbon dioxide at that place, and that, since the gradients of oxygen and carbon dioxide concentrations are steep, the respiratory activity falls rapidly from the surface inwards. These considerations explain the very much higher rates of respiration in tissue slices as compared with whole organs. Since the high respiratory activity of a thin slice of tissue takes some time to develop after it is cut out of the organ, it is suggested that oxygen is necessary to activate either substrate or enzyme system.

In nitrogen tissue slices of beetroot, mangold root, potato tuber, and artichoke show a behaviour as regards carbon dioxide output similar to that of whole organs. Accepting Blackman's scheme for the breakdown of carbohydrate under aerobic and anaerobic conditions, there is evidence of oxidative anabolism in all the tissues examined. It is shown that Lipmann's finding of an inhibition of carbon dioxide output in yeast juice by oxygen in presence of oxidation-reduction systems is not inconsistent with Blackman's theory.

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Studies in the Physiology and Morphology of *Penicillium notatum*

I. Production of Penicillin by Germinating Conidia

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With two Figures in the Text

INTRODUCTION

IN view of the therapeutic importance of penicillin, the emphasis in mycological work has so far been laid on the large-scale culture of *Penicillium notatum* rather than on more fundamental questions. Various workers have described the general course of fungal growth during the production of penicillin and the influence on production of certain factors, in particular of the pH of the medium (e.g. Fleming, 1929; Abraham et al., 1941; Challinor, 1942; Kocholaty, 1942; Foster, Woodruff, and McDaniel, 1943; Dimond and Peltier, 1945; Pratt, 1945), but little precise information is available as to the biochemical processes ultimately involved.

The production of penicillin by *P. notatum* may be associated with the growth of hyphal tips, with the elongation of cells behind the growing point, with the metabolism of mature cells, or with the autolysis of the older, senescent regions. The available data indicate an increasing rate of production as the fungal colony develops. Pontecorvo (1945) states that the maximum rate of secretion in a well-grown colony lies in the region approximately 1 cm. behind the outer margin; the nature of the producing cells still awaits investigation.

The present investigation has been undertaken to ascertain which region (or regions) of the hyphae is involved in penicillin production. This paper specifically relates to the question: 'What is the earliest developmental stage at which evidence of penicillin production can be obtained?' It was considered that the information which might accrue from such an investigation would not only be itself of a fundamental nature, but would make possible a more precise interpretation of data on the metabolic processes involved.

MATERIALS AND METHODS

The method employed was based on that used by Brown (1915, 1917) in enzymological studies of hyphal tips. It consisted of: (i) harvesting large numbers of conidia from stock cultures; (ii) washing these and inoculating them into small volumes of a medium suitable for the production of penicillin;

(iii) assaying for penicillin at regular intervals; (iv) examining microscopically the germinating conidia.

The strain of *P. notatum* used was the industrial strain described as NRRL.1249.B21. Stock cultures were grown in 16-oz. 'medical flats' on the following sporulation medium which was based on that of Moyer and Coghill:

Sporulation medium		Solution A	
Glycerol	10 gm.	KH ₂ PO ₄	3 gm.
Molasses	10 "	Distilled water to 1 litre	
Peptone	5 "	Solution B	
Solution A	20 c.c.	NaCl	250 "
" B	20 "	MgSO ₄ ·7H ₂ O	2·5 "
Distilled water to 1 litre		FeSO ₄ ·9H ₂ O	0·6 "
pH adjusted to 6·5 with NaOH		MnSO ₄	0·2 "
Agar	2 per cent.	CuSO ₄ ·5H ₂ O	0·2 "

Conidia were harvested after 6–9 days and washed twice to remove any penicillin present. It was shown that the water from the second washing was completely inactive when tested against the bacteria used for assaying the medium. The conidia were germinated in a corn-steep medium based on that of Moyer and Coghill having the following composition:

Corn-Steep Medium

Corn-steep liquor	10 per cent.
Lactose	2 "
Glucose	2 "
NaNO ₃	0·3 "

In the technique first employed shallow layers of the medium in conical flasks were inoculated. This proved unsatisfactory as the percentage of germination was low, due apparently to the sinking of the conidia. A modification of the technique of Fleming and Smith was finally adopted, a dense conidial suspension being spread on the surface of a disc of permeable cellophane. This rested on a layer of glass wool holding 7·5 c.c. of the corn-steep medium in a Petri dish. At intervals the cellophane was removed from duplicate dishes, the germinating conidia were fixed for microscopic examination, and the medium was drawn off and assayed for penicillin.

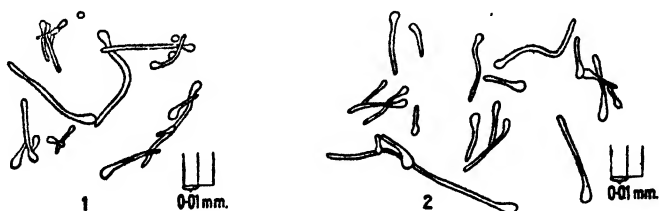
Two methods of assay were used: (i) the Oxford cup method, using *Bacillus subtilis* as the test organism (see Abraham et al., 1941; Foster and Woodruff, 1943 and 1943a); (ii) serial dilutions of the liquid in a nutrient broth were inoculated with *Staphylococcus aureus*, incubated and examined for turbidity (see Fleming, 1929; Foster and Woodruff, 1943). These methods will be referred to as the Oxford cup method and the turbidity method. Penicillin concentrations are given in Oxford units (O.u.). It should be noted that the use of the term 'penicillin' in this paper involves the assumption that the antibiotic substance detected was in fact penicillin.

Concurrently, certain additional morphological observations of the course of germination were made, the conidia being inoculated either into a hanging

drop of corn-steep medium or into one end of a thin streak on a coverslip of this medium with 0.5 per cent. agar.

RESULTS AND OBSERVATIONS

In a preliminary experiment medium assayed by the Oxford cup method 12 hours after inoculation gave small inhibition rings, the penicillin concentration, however, being too low for quantitative estimation by this method.



FIGS. 1 and 2. Germinating conidia. Fig. 1, 11 hours after inoculation; Fig. 2, 12 hours after inoculation.

In a further experiment duplicate samples of the medium were tested for penicillin by the turbidity method 9, 10, 11, 12, 13½, and 15 hours after inoculation, with the results shown in the table below.

TABLE

+ indicates heavy turbidity. T indicates trace of growth.
R „ reduced turbidity. — „ no visible growth.

Liquid tested.	Volume of liquid in c.c. added to 10 c.c. of test medium.					
	0.8	1.0	1.2	1.4	1.6	1.8
0.2 O.u./c.c.	+	+	T	T?	—	—
standard penicillin solution	+	+	T	T?	—	—
9-hr. medium			+	+	+	+
10-hr. medium			+	+	+	+
11-hr. medium	+	+	+	+	+	+
12-hr. medium	+	+	R	R	R	R
13½-hr. medium (½ dilution)	+	+	R	T	R	
15-hr. medium (½ dilution)	T	T	—	—		
	T	T	T	T		

These observations were confirmed in a third experiment, in which tests were made by the Oxford cup method on duplicate samples of medium at periods of 10 (1 sample only), 11, 12, 13, 14, and 15 hours after inoculation. Again small inhibition rings were obtained from 12 hours onward.

Figures 1 and 2 illustrate representative samples of the germinating conidia from the second experiment after 11 and 12 hours respectively. These figures

are characteristic of the stage of development reached after these periods in all experiments, the average length of the germ tubes being approximately 30μ .

Additional morphological observations made on small aggregates of germinating conidia have made it clear that germination and development are influenced by the proximity of other conidia. For example, growth is more rapid in the case of germ-tubes from groups of conidia than in those from single ones; on the other hand, the presence of closely adjacent parallel hyphae appears to reduce the number of branches formed by a particular hypha. These observations, which have provided background information for the present investigation, will be considered more fully in a later paper.

DISCUSSION

The results obtained show that the young terminal regions of the hyphae, averaging 30μ in length, produce penicillin. Whether this production is associated with the actual tip or with the region immediately behind it or with both cannot be decided from the data. It may be noted that even the densest cultures of newly germinated conidia have yielded no evidence of the presence of penicillin; this may be due either to production not having begun or to the concentration in the medium being too low for detection.

The results so far obtained leave it an entirely open question as to the extent to which the older regions of the hyphae may contribute to the production of penicillin. The rate of production may be associated solely with the multiplication of the young terminal regions; alternatively, as the colony grows, the increase in numbers of older cells, as well as of young branches, may promote the process. The extension of this investigation will therefore require concurrent studies of morphological development and of the course of penicillin production.

The observations on the influence which germinating conidia exert on one another make it clear that information obtained from small groups of conidia will not apply to the dense aggregates used in the experiments already described. Except in the very early stages of germination the microscopic examination of such aggregates presents considerable difficulties. It is therefore desirable that the penicillin production of small numbers of conidia germinating in correspondingly small volumes of medium should be studied. The assay of very small amounts of liquid is at present being explored.

SUMMARY

In a preliminary investigation to ascertain with which region (or regions) of the hyphae of *Penicillium notatum* penicillin production is associated, it has been found that the presence of penicillin can be demonstrated in a suitable medium 11–12 hours after inoculation with conidia. At this stage only young germ-tubes, approximately 30μ in length, are present. The extent to which older regions of the hyphae are involved in penicillin production awaits further investigation.

The writer wishes to thank Professor C. W. Wardlaw, under whose supervision this work was carried out, for his valuable help and criticism. She is indebted to Imperial Chemicals (Pharmaceuticals) Ltd. for a grant which made the work possible.

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Comparative Physiological Studies on the Growth of Field Crops

I. Variation in Net Assimilation Rate and Leaf Area between Species and Varieties, and within and between Years

BY

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With five Figures in the Text

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INTRODUCTION

FIELD experiments carried out in the past century have provided much information on the relation between crop yield and environmental conditions, especially those which can be controlled by husbandry operations. The interpretation of the empirical results of field experiments is not easy, because an apparently simple cultural operation such as the application of a fertilizer may produce complex changes in the environment. When the nature of these changes is known, there remains the problem of how they have affected the physiology of the plants. An explanation can often be suggested from physiological principles established by laboratory studies, but this procedure has obvious limitations. The plant material used in physiological research is usually selected on grounds of experimental convenience, and not on its economic importance, so that application of the results to agricultural crops may involve the assumption that different species show the same physiological behaviour. The experimental variation in a factor investigated in the

laboratory may be outside the range encountered in the field, and the effects of environmental factors vary with change in their intensity. Laboratory investigations are usually concerned with an isolated aspect of physiology, and the way in which different processes interact is little understood. Even if it is shown that a change in the environment produces an effect on some particular physiological process, it is difficult to assess the importance of the effect in determining the change in growth and yield. For example, although deficiency in the supply of potassium is known to reduce the rate of photosynthesis of the leaves of some species of plants at certain stages of growth, it would be rash to assume that this is sufficient to account for an increase of yield produced by application of potassic fertilizer to a field crop. Other effects may be of much greater significance. It follows that, for a physiological analysis of the causes of variation of crop yield, laboratory studies must be supplemented by direct investigation of crops growing in field conditions.

Techniques which involve complicated measurements are not practicable for investigations on field crops, because the high variability in the plant population necessitates the repetition of each observation on a large number of samples. The methods of growth analysis developed by Gregory (1917) and West, Briggs, and Kidd (1920) are particularly suitable for field investigations, for they enable growth functions of definite physiological significance to be calculated from simple measurements of the dry weight and leaf area of plants made at intervals throughout growth. One of these growth functions, the net assimilation rate (unit leaf rate of West, Briggs, and Kidd), defined as the rate of increase of dry matter per unit area of leaf surface, is of special interest, for it is a measure of the rate of photosynthesis in the leaves, less the respiratory loss of dry matter in the whole plant. Since, by definition, the increment of dry matter in any interval of time is the integral of the product of net assimilation rate and leaf area, the progress of dry-matter accumulation can be completely described in terms of the changes in the two attributes, net assimilation rate, which measures the intensity of carbon assimilation, and leaf area, which is a measure of the size of the assimilating system. This is a useful form of analysis, since it isolates one simple component, the net assimilation rate, leaving leaf area as the integrated measure of all the other complexities of growth and differentiation. Further analysis involves a study of the factors which control leaf area, distinguishing between effects on leaf number and on the size of individual leaves.

It should here be noted that dry-matter accumulation in a crop is only a partial measure of agricultural yield. Usually such yield refers only to one morphological or chemical component of the plant, for example, the tubers of potatoes, the grain of cereals, the fibre of flax, or the sucrose in the root of sugar-beet, the remaining parts being of less or of no economic value. The full analysis of yield requires consideration of the distribution of dry matter between different parts of the plant, as well as of the total weight of dry matter accumulated. However, this is of secondary importance, for the weight the component parts are usually highly correlated with the total dry

of the plant. Variation in the partitioning of assimilate between different organs is rarely sufficiently great to offset variation in total dry weight.

In the work to be discussed in this series of papers the method of growth analysis has been used to investigate the physiological causes of variation in the yield of field crops. In particular, the object has been to relate variation in dry-matter production to changes in net assimilation rate and the total leaf area of the plant. The present paper deals with the variation of net assimilation rate and of leaf area throughout the growing period, in different crops and years.

Although the appropriate methods of growth analysis were formulated nearly 30 years ago, little use has been made of them in agricultural or ecological research. Heath and Gregory (1938) summarized the available data on net assimilation rate, mostly derived from pot cultures, but including some from field crops. They compared the net assimilation rates of different species by means taken over periods ranging from 4 to 14 weeks, in order to eliminate the effects of short-period variations in meteorological conditions, and concluded that the mean net assimilation rate during the vegetative phase was approximately constant for all the diverse plants and environments investigated; 'on the average, despite variations in leaf structure, the net amount of CO_2 utilized per unit area is everywhere the same'. They also point out that if net assimilation rate tends to a constant value under certain conditions, variations in dry-matter accumulation must be determined mainly by differences in leaf area.

Gregory (1926) and Heath (1937) have shown, in barley and cotton respectively, that net assimilation rate is constant with time, in the early stages of growth up to the time of the beginning of flowering, apart from fluctuations due to changes in meteorological conditions. The inference is that the internal factors controlling net assimilation rate are independent of the age of the plant during the vegetative phase. Ballard and Petrie (1936) and Williams (1937, 1939) have presented data for wheat, Sudan grass, and oats which do not agree with this conclusion.

MATERIAL AND METHODS

Observations were made, as opportunity occurred, on a variety of crops grown in 15 field experiments on the Rothamsted farm in 6 years between 1933 and 1943. A full account of one of these has already been published (Watson and Baptiste, 1938; Watson and Selman, 1938), but the relevant results are included in this paper for purposes of comparison. This experiment compared two crops, sugar-beet and mangolds, but in the others only a single crop was grown. The crops investigated were wheat, barley, sugar-beet, mangolds, and potatoes. Cultural details for each experiment are given in Table I. The experimental treatments included comparisons of varieties and sowing dates, and tests of fertilizers. The present paper is mainly concerned with the means of all treatments, but some reference is made to varietal differences and to the effects of varied sowing date.

TABLE I
Details of Experimental Crops

Harvest year.	Experiment no.	Crop.	Variety.	Date of sowing or planting.	Number of plots.	Size of Sample	
						A.	B.
1934	1	Wheat	Victor	Oct. 12	64	128	64
	2	Sugar-beet	Kleinwanzleben E	Six dates between Apr. 9 and June 18	6	120	60
		Mangold	Yellow Globe		6	120	60
1937	3	Wheat	Squarehead's Master Yeoman Victor	Oct. 23	24	96	48
	4	Barley	Plumage Archer	Apr. 23	8	32	16
	5	Sugar-beet	Kleinwanzleben E	Apr. 30	18	270	90
			Dobrovice N	May 25			
			Marsters	June 12			
1938	6	Potatoes	Ally	May 27	12	180	60
	7	Wheat	Squarehead's Master Yeoman Victor	Nov. 10	24	96	48
	8	Wheat	Victor	May 20	4	40	16
	9	Sugar-beet	Kleinwanzleben E	May 13	10	150	50
			Dobrovice N	May 27			
1939	10	Potatoes	Marsters Ally Arran Banner British Queen Great Scot Majestic	Apr. 29	15	225	50
	11	Wheat	Red Standard	Oct. 25	14	112	112
	12	Barley	Plumage Archer	Mar. 10	10	100	100
	13	Mangold	Yellow Globe	May 9	14	280	140
1942	14	Sugar-beet	Kleinwanzleben E	Apr. 25	48	288	—
1943	15	Sugar-beet	Kleinwanzleben E	Apr. 14	48	288	—

NOTES

Column A shows the total number of metre-lengths of drill row of cereals, or of plants of the root crops taken from the experiment on each sampling occasion.

Column B shows the total number of leaves of the root crops, or of shoots of the cereals, for each of which fresh weight and lamina areas were determined, for the estimation of leaf area per sample. In experiments 14 and 15 leaf area was determined by a different method (see text, p. 47).

Experiment 1. An experiment on the effect of time of application of nitrogenous fertilizer. (For details see Rothamsted Annual Report, 1934, p. 181.)

Experiment 2. For a full account of this see Watson and Baptiste (1938).

Experiments 3, 4, 5, 6, 7, 9, and 10. These were laid down for sampling observations in connexion with the Crop Weather Scheme of the Agricultural Meteorological Committee. The experimental treatments consisted only of comparisons of varieties and sowing dates. In experiments 4 and 6 all plots were uniformly treated. The wheat and barley had top-dressings of 1-1½ cwt. of sulphate of ammonia per acre. The root crops had a complete dressing of fertilizers, and the potatoes had dung in addition. The quantities varied and are not given in detail as they are not relevant

to this paper. In the sugar-beet experiment all three varieties were sown on each sowing date. In experiment 9 the second sowing of Marsters failed.

Experiment 8. This consisted of four uniformly treated plots of a winter variety of wheat sown in late spring, on about the same date as the sugar-beet of experiment 4.

Experiment 11. The Broadbalk experiment.

Experiment 12. The Hoosfield experiment.

Experiment 13. The Barnfield experiment.

These are three of the classical experiments of Lawes and Gilbert on which the same crop has been grown continuously on the same site for a long period of years. For a full account of their history see A. D. Hall, 'The Book of the Rothamsted Experiments', John Murray, London, 1905.

Experiments 14 and 15. These were the first two crops of a new rotation experiment on the effect of frequent applications of agricultural salt and muriate of potash. The treatments consist of varying rates of these fertilizers, applied alone or in combination. All plots received a uniform dressing of sulphate of ammonia and superphosphate.

The experiments were not designed specifically for the purpose of growth analysis. Many of them were laid down in connexion with a scheme of sampling observations on field crops which formed part of the Crop Weather Scheme of the Agricultural Meteorological Committee.¹ Simple measurements, mostly of size attributes of the plant and of the density of the plant population, were made at intervals during growth, with the object of discovering correlations between attributes of growth and final yield which could be used for the purpose of crop forecasting. By supplementing the observations scheduled under this scheme with determinations of dry weight and leaf area, data appropriate for growth analysis were conveniently obtained. Other experiments were primarily yield trials, and in these the sampling observations were restricted to a small area of each plot, or of selected plots, the remainder being left undisturbed for the measurement of yield by ordinary harvesting methods. In one year, 1939, three of the classical field experiments begun by Lawes and Gilbert were used in this way.

Observations were usually made at intervals of a fortnight. In a few cases the interval was a week, and for those experiments in which only a small part of each plot was available for sampling, longer intervals, of 3 or 4 weeks, had to be allowed between successive observations in the later stages of growth.

The size of samples taken was determined primarily by the amount of labour available for making the required measurements. Usually 4 to 6 workers took part in the work. On each occasion the field operations were completed within 1 day, except for the larger experiments which occupied 2 days. At least 2 independent samples, each consisting of 1 or more metre-lengths of drill row of a cereal crop and 5 or more adjacent plants of a root crop, were taken at random from every plot on each occasion. The total bulk of plant material handled at each sampling ranged from 100 to 300 plants of sugar-beet, man-golds, or potatoes, and from 30 to 100 metre-lengths of drill row of the cereal crops.

¹ A joint committee of the Ministry of Agriculture and Fisheries, the Department of Agriculture for Scotland, the Meteorological Office, and the Forestry Commission.

The samples from the cereal crops were based on a unit area of crop, definable by a length of drill row since the spacing between the rows was constant (6 in.); plant number per sample was variable. The root-crop samples, on the other hand, contained a constant number of plants and represented varying areas of crop, because the spacing between plants in the row varied. This difference in the basis of sampling was dictated by convenience. As soon as tillering starts it becomes difficult to distinguish between individual plants in a cereal crop, while if the size of sample of a widely spaced crop such as potatoes is specified by a length of row, it is frequently doubtful whether a plant should be included in the sample or not. It is unlikely that the estimates of net assimilation rate were much affected by the difference in the sampling basis. The average spacing of the plants in the root crops was determined by counting the total number of plants on each plot, and this allowed measurements made on the samples to be expressed on the basis of area of crop, when this was desired.

The procedure of sampling was similar to that already described for sugar-beet and mangolds (Watson and Baptiste, 1938). The following observations were made:

(1) *Fresh weight per sample*, recorded separately for leaf lamina, petiole (as defined by Watson and Baptiste, 1938), and root in the sugar-beet and mangold samples. For potatoes, tubers and haulms (including rhizomes and roots) were weighed separately and the weight of leaf lamina was determined on one plant per sample. No separations were made on the barley and wheat samples.

(2) *Dry weight per sample*, determined on the separate parts of the plant, as for fresh weight. In the early stages of growth the whole of each sample was dried; later when the bulk of material became very large the dry-matter content was determined on sub-samples, and the total dry weight was estimated from dry-matter content and fresh weight. Drying was carried out in a large electric oven with ventilation for one day at 70–100° C. and for a second day without ventilation at 100° C.

(3) *Plant and shoot number per sample*, for the cereals, and *leaf number per sample* for the root crops. Leaves were counted on only one plant, selected at random, in each sample of potatoes. Leaves shorter than 1 in. in length and dead leaves were excluded from the counts.

(4) *Leaf area per sample*. For the root crops, observations (1) and (3) provided an estimate of mean fresh weight of lamina per leaf. The lamina area: lamina fresh weight ratio and its regression on lamina fresh weight were estimated from a small subsidiary sample of leaves. The mean area per leaf and per sample was computed from these measurements by the method previously described (Watson, 1937). The subsidiary sample consisted of a random selection of about 10 leaves for each experimental treatment tested in the experiment. A 'blue-print' was made of each leaf lamina, and its area was measured later by planimeter.

The leaf area per sample of the cereal crops (excluding the leaf-sheath) was

determined similarly, except that it was based on the mean fresh weight per shoot, determined from observations (1) and (3), and the mean ratio of lamina area per shoot : shoot fresh weight and its regression on shoot fresh weight, estimated from a subsidiary sample of randomly selected shoots.

A different method was used in the sugar-beet experiments of 1942 and 1943. All the detached leaf laminae from the sample were piled at random in a thick layer on a large wooden tray, and discs of known area were cut out with a circular punch of about 1 in. diameter. A sample of these discs, usually 100 gm., was weighed and the number of discs counted. In this way an estimate of the lamina area : lamina fresh weight ratio appropriate to each sample was obtained, and this, multiplied by the fresh weight of leaf lamina in the sample, gave the total leaf area per sample.

The net assimilation rate was estimated by the formula

$$\frac{W_2 - W_1}{t_2 - t_1} \times \frac{\log_e L_2 - \log_e L_1}{L_2 - L_1},$$

where W_1 and W_2 are the total dry weights per sample and L_1 and L_2 the leaf areas per sample, at times t_1 and t_2 respectively. Williams (1946) has pointed out that this formula gives an accurate measure of mean net assimilation rate over an interval, that is, of the mean value of $\frac{1}{L} \frac{dW}{dt}$, only if the relation between W and L is linear. The deviations from linearity in the relation between W and L over the short intervals used are sufficiently small to justify the use of the approximate formula.

Throughout the remainder of this paper net assimilation rate is referred to as N A R, and is expressed as grammes per sq. decimetre of leaf area per week.

N A R was calculated for individual plots of each experiment, and the values given in the following graphs and tables are arithmetic means for all plots. This procedure, though laborious, enabled estimates of the standard errors of the means to be computed by the analysis of variance method. The arithmetic means differed little from weighted means of N A R computed from the mean dry weights and leaf areas of all samples on each occasion. The weighted means can be calculated with much less labour than the arithmetic means, and are perhaps better measures of the average behaviour of the crop, but their standard errors cannot be computed directly.

One difficulty encountered in working with field crops is that the whole of the root system cannot be recovered. For the cereal crops and potatoes all the fibrous roots dug up with the samples were included in the total dry weight, but the fibrous roots of sugar-beet and mangold were lost during the removal of soil from the storage root by washing. In one experiment on wheat, N A R was calculated for total dry weight including all the roots recovered, and from the dry weight of the shoots alone. The estimates by the two methods differed only slightly, and this suggests that errors in the determination of N A R due to incomplete recovery of roots were small.

RESULTS

*Part I. Net Assimilation Rate**Seasonal variation*

The changes with time in the N A R of each of the 16 crops are shown in Fig. 1, where the mean value for each sampling interval is plotted at the mid-point of the interval. The vertical lines at the side of each graph represent the magnitudes of differences between means for different intervals which are significant at the 5 per cent. level. These are given by $\sqrt{2t} \times$ standard error of the means, where t has the value appropriate to the number of degrees of freedom on which the estimate of the standard error is based. The standard errors were estimated from the pooled error variances for all sampling intervals of an experiment, and as the error variance tended to increase in the later stages of growth, the significant differences shown overestimate the true values for the earlier intervals, and underestimate those for the later intervals.

The designs of the experiments which supplied the material for the 1939 results do not allow of the calculation of valid estimates of error for the effects of manurial treatments applied to the different plots, owing to the lack of replication and the systematic arrangement of the treatments, but an estimate of error of the means of all plots for successive sampling intervals can be computed from the variance within plots between intervals, and this has been used to calculate the significant differences shown in Fig. 1. In so far as effects of manurial treatment on N A R varied with time of sampling, the significant differences so determined are overestimates of the true values.

The graphs in Fig. 1 do not cover the whole of the growth period of each crop. Those for wheat and barley are continued only up to the beginning of ear emergence. After the ears have emerged, photosynthesis in the ear itself accounts for a considerable fraction of the dry matter subsequently accumulated (Boonstra, 1929; Smith, 1933; Watson and Norman, 1939). Values for N A R calculated for periods after ear emergence therefore give incorrect estimates of the N A R of the leaves, for they refer the whole dry-matter increase to the leaves, including that attributable to the ears. In the few cases in which measurements of leaf area and dry weight were continued after ear emergence, it was found in accordance with expectation that the apparent N A R rose to much higher values than those in the period immediately before ear emergence. The estimates of N A R before ear emergence are probably slightly too high because assimilation in the leaf sheath is referred to the leaf lamina. The records for wheat in 1933-4 began on November 23 soon after germination was completed and continued throughout the winter and spring until the time of ear emergence. The data for the wheat crops of 1937, 1938, and 1939 cover only the period May-June. Observations on the potato crops began in mid-July after the early inter-row cultivations were completed. It was found impossible to devise a satisfactory sampling procedure for the earlier period, because the repeated grubbing and earthing-up operations alternately exposed and buried the young plants. In 1934, 1939,

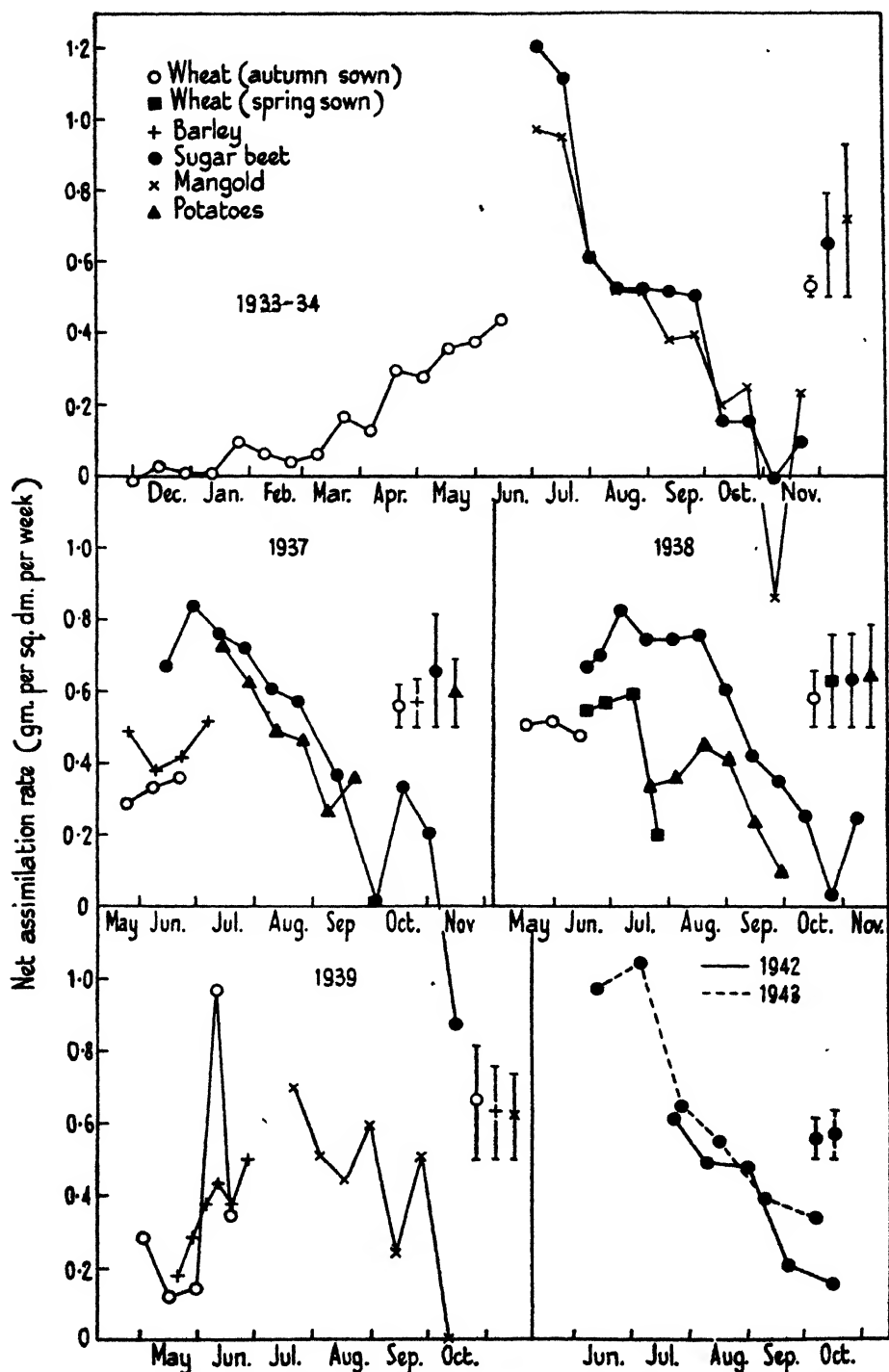


FIG. 1. Change with time of net assimilation rates of different species. The vertical lines represent significant differences between means for different intervals.

and 1942 sampling of the sugar-beet and mangold crops began soon after singling was completed; the records for the other years 1937, 1938, and 1943 include some observations in the period before singling.

The most obvious feature of the graphs in Fig. 1 is that N A R varied throughout the year in a similar manner in all the years investigated, rising during the spring from low winter values to a maximum at the end of June and subsequently falling during the late summer and autumn. This is most clearly seen in the results for 1933-4 which cover the whole of a growing season. Here the rising and falling phases are represented in different species, but there is evidence from other years that the upward trend of the N A R of wheat and the downward trend of that of sugar-beet were not determined by factors specific to the species, for in 1937, 1938, and 1943 part of the phase of rising N A R is recognizable in the observations on sugar-beet, which were begun earlier than in 1934. In all these years N A R rose to a maximum at the end of June and subsequently fell. A similar drift with time in the N A R of sugar-beet was found by Boonstra (1939).

Although the observations on wheat and barley in 1937, 1938, and 1939 covered only the short period, from May to June, they show a rising trend similar to that of wheat in 1934. The records for potatoes in 1937 and 1938, extending over the period July to September, show a decline of N A R with time, like that found for sugar-beet. In 1938 there was a preliminary rise of N A R of potatoes to a maximum in the middle of August. A similar peak in mid-August is apparent in the graph for sugar-beet, but the value of N A R for sugar-beet at this time was below the maximum at the end of June.

There can be little doubt that the fluctuation of N A R throughout the year from low winter values to high values in midsummer is related to seasonal change in meteorological conditions; the maxima and minima of N A R correspond in time with those of day length, temperature, and radiation. Superimposed on this seasonal drift of N A R, more rapid fluctuations are apparent in Fig. 1, of which the rise in the N A R of potatoes and sugar-beet in July-August 1938, referred to above, is an example. These may be attributable in part to experimental error, but the possibility that they are related to short-period changes in weather conditions is discussed later.

Variation from year to year

The variation between years in N A R for each species is shown in Table II, which gives mean values of N A R taken over the longest calendar periods common to all the years investigated. In computing the means the values for each sampling interval were weighted according to the length of the interval, in the experiments in which this was variable. The standard errors attached to the means were computed from the error variance between plots in each experiment, and the use of these standard errors to test the significance of differences between the means is, therefore, a test of the null hypothesis that the variation between crops grown in different years and in different fields was not greater than the variation between plots in the same field. That is to

say, differences between the means cannot be attributed wholly to differences between years in climatic conditions, but may be due in part to differences between fields. For a rigid test of annual variation, observations on crops grown on the same site in similar conditions over a period of years, or on a number of crops in different fields each year, are necessary. For reasons already stated no standard errors can be given for the 1939 results.

TABLE II

Net Assimilation Rates (gm. per sq. dm. per week) of the same Species in Comparable Periods of Different Years

		Interval.	Mean N A R.
Wheat	1934	May 10-June 21	0.39 ± 0.017
	1937	May 18-June 28	0.32 ± 0.020
	1938	May 9-June 20	0.50 ± 0.018
	1939	May 9-June 20	0.30
Barley	1937	May 19-June 29	0.42 ± 0.017
	1939	May 16-June 29	0.36
Potatoes	1937	July 7-Sept. 28	0.49 ± 0.028
	1938	July 14-Sept. 22	0.36 ± 0.032
Sugar-beet	1934	July 10-Oct. 30	0.51 ± 0.012
	1937	July 19-Oct. 25	0.42 ± 0.024
	1938	July 12-Nov. 1	0.49 ± 0.026
	1942	July 16-Oct. 30	0.35 ± 0.008
	1943	July 16-Oct. 20	0.46 ± 0.010
Mangold	1934	July 10-Oct. 30	0.48 ± 0.012
	1939	July 14-Oct. 18	0.43

TABLE III

Net Assimilation Rates (gm. per sq. dm. per week) of Different Species in Comparable Periods of the Same Year

		Interval.	Mean N A R.
1937	Wheat	May 18-June 28	0.32 ± 0.020
	Barley	May 19-June 29	0.42 ± 0.017
1939	Wheat	May 9-June 20	0.30
	Barley	May 16-June 22	0.33
1937	Wheat	May 31-June 28	0.34 ± 0.025
	Sugar-beet	June 7-July 5	0.75 ± 0.057
1938	Wheat (spring sown)	June 14-Aug. 3	0.46 ± 0.017
	Sugar-beet	June 14-Aug. 9	0.75 ± 0.027
1938	Wheat (spring sown)	July 19-Aug. 3	0.20 ± 0.081
	Potatoes	July 14-July 28	0.34 ± 0.023
1937	Barley	June 1-July 13	0.44 ± 0.030
	Sugar-beet	June 7-July 19	0.75 ± 0.056
1937	Barley	June 29-July 13	0.51 ± 0.080
	Potatoes	July 7-July 21	0.75 ± 0.056
1934	Sugar-beet	July 10-Nov. 28	0.41
	Mangold		0.37 } ± 0.011
1937	Sugar-beet	July 5-Sept. 27	0.56 ± 0.026
	Potatoes	July 7-Sept. 28	0.49 ± 0.028
1938	Sugar-beet	July 12-Oct. 4	0.60 ± 0.030
	Potatoes	July 14-Oct. 6	0.31 ± 0.029

It is clear from Table II that N A R was not constant from year to year. The mean N A R of wheat for the period May–June ranged in different years from 0.3 to 0.5 gm. per sq. dm. per week; the means for sugar-beet in the period July–October and potatoes in the period July–September had a similar range, from 0.35 to 0.5. For all three species of crop the variation between years was significantly greater than the variation between similarly treated plots in the same field, of which the standard errors provide a measure.

Table II gives no conclusive evidence on the question of whether the N A Rs of different species were affected similarly by variations in climatic conditions in different years. Only two comparisons between the same years for different species can be made. The mean N A R during May–June of both wheat and barley was greater in 1937 than in 1939. On the other hand, the mean N A R of sugar-beet during July–October was less in 1937 than in 1938, while that of potatoes during July–September was greater in 1937 than in 1938.

The observations on sugar-beet and mangolds cover a long period in each of the years investigated, and it is of interest to compare the drifts of N A R with time, as well as the mean values, in these years. This was done by fitting a linear regression on time to all values of N A R available each year in the period between the maximum at the end of June and the end of October. The linear regression coefficients in gm. per sq. dm. per week per week, which measure the mean rate of change of N A R with time, were as follows:

	Sugar-beet.		Mangold.
1934	– 0.061 ± 0.0066	1934	– 0.048 ± 0.0096
1937	– 0.043 ± 0.0063	1939	– 0.041 ± 0.0078
1938	– 0.048 ± 0.0059		
1942	– 0.040 ± 0.0042		
1943	– 0.048 ± 0.0047		

The negative sign of the coefficients indicates that N A R fell with time. All the coefficients were highly significant; this merely provides arithmetical confirmation of the reality of the downward trend of N A R which is obvious in Fig. 1. The variation between the coefficients for different years was not great compared with the magnitude of the standard errors, and only the extreme difference, that between the coefficients for sugar-beet in 1934 and 1942, was significant.

The fitted regression lines are plotted in Fig. 2. The lines for different years are most widely separated in June and July. As the season advances the lines converge to very low values in October. Thus the differences between years in N A R were greatest in midsummer when N A R had its highest values, and later in the season the differences between years diminished. It follows that the rate of fall of N A R with time was high in years when the mean N A R were high. That this was so can be seen by comparing the regression coefficients with the mean values of N A R given in Table II.

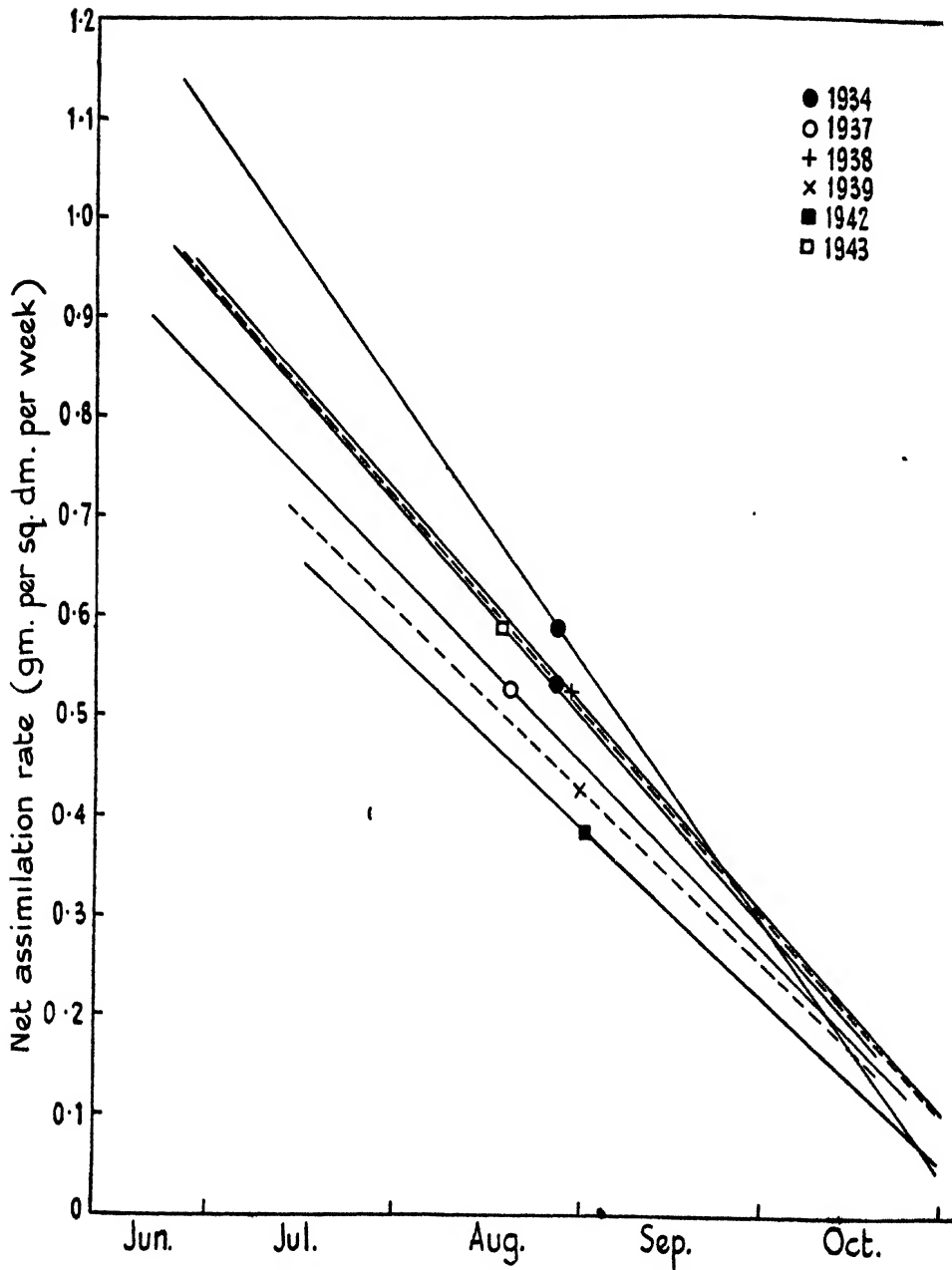


FIG. 2. Linear regression lines of net assimilation rate of sugar-beet (full lines) and mangold (dotted lines) on time, in different years.

Differences between species

All the possible comparisons of N A R which can be made between different species in similar periods of the same year are shown in Table III. Species were taken in pairs, and for each of the pair the mean N A R was calculated for the longest period of time common to the observations on both. The standard errors of the means were computed from the error variance between plots, as in Table III, so that differences between the means judged to be significant by comparison with the standard errors cannot be attributed wholly to differences between species, but may be partly the result of variation between fields. However, the differences between species were consistent from year to year, and this suggests that the effect of inter-field variation was not great.

In both 1937 and 1939 the mean N A R of wheat during May and June was less than that of barley, though the difference in 1939 was small. Fig. 1 shows that the difference between the means for 1939 was greatly affected by one very high value of N A R recorded for wheat in the interval June 6–13. For the other intervals in 1939 there was a greater difference between wheat and barley than that indicated by the means in Table III.

The N A R of sugar-beet was more than twice that of autumn-sown wheat in June 1937. It also greatly exceeded that of barley (1937) and spring-sown wheat (1938) in June and July. Comparison of autumn-sown wheat with sugar-beet in 1934 and 1938 leads to the same conclusion, although in these years the records for the two species did not overlap. The latest observations on the wheat before ear emergence gave values of N A R much below the earliest observations on sugar-beet.

When sugar-beet and mangold were compared in the same experiment in 1934 (Watson and Baptiste, 1938) the mean N A R of sugar-beet in the period July–November was found to be slightly greater than that of mangold. These results are included in Table III and Fig. 1.

Sugar-beet also had a higher N A R than potatoes in both 1937 and 1938. The difference was fairly constant throughout the period July–September in 1937 (Fig. 1), but in 1938 it was particularly large in the early stages of growth in July and August. Later the difference diminished, though it continued to be greater than in 1937.

It is possible to compare potatoes with the cereals only for single sampling intervals of a fortnight, and the dates do not correspond precisely for the two crops. In these short periods in July potatoes had a markedly greater N A R than barley in 1937 and spring-sown wheat in 1938.

These results lead to the conclusion that N A R varies from species to species. The most outstanding contrast is that between the root crops and the cereals, and this confirms the results of Müller (1904) who compared the apparent assimilation rates (dry-weight increase per unit area, corrected for translocation but not for respiration) of the leaves of a range of species by means of the 'half-leaf' method, and concluded that the assimilation rate of dicotyledons was greater than that of monocotyledons.

TABLE IV

Mean Net Assimilation Rates (gm. per sq. dm. per week) of Different Varieties of the Same Species

WHEAT							
				1937	1938		
Interval				May 18–June 28	May 9–June 20		
Variety:							
Squarehead's Master				0·32	0·48		
Yeoman				0·32	0·49		
Victor				0·33	0·53		
S.E.				±0·035	±0·031		

SUGAR-BEET									
				1937	1938				
Interval				July 5–Oct. 25	June 28–Oct. 18				
Sowing date . . Apr. 30				May 25 June 12 Mean	May 13	May 27	Mean		
Variety:									
Kleinwanzleben E 0·44				0·50	0·55	0·50	0·62	0·58	0·60
Dobrovice N . 0·38				0·43	0·50	0·44	0·52	0·58	0·55
Marsters . . . 0·51				0·51	0·50	0·51	0·63	—	—
S.E.				±0·036	±0·021	±0·037	±0·026		

POTATOES							
				1938			
Interval				July 14–Aug. 25	Aug. 25–Oct. 6		
Variety:							
Ally				0·45	0·12		
Majestic				0·41	0·34		
Arran Banner				0·38	0·25		
Great Scot				0·37	0·34		
British Queen				0·30	0·18		
S.E.				±0·012	±0·130		

Varietal differences

A number of the commonly grown varieties of wheat, sugar-beet, and potatoes were included in the experiments of 1937 and 1938, primarily with the object of ensuring that the results should have general application. The mean N A Rs of the different varieties are compared in Table IV. The differences between varieties shown in this table, unlike the differences between years and species in Tables II and III, are independent of variation between fields, for the comparisons were made within the same experiment. The standard errors are strictly appropriate for testing the varietal differences. ✓ There was no evidence of any differences in N A R between varieties of wheat. The means for the three varieties in May–June 1937 were almost identical, and the slight superiority of Victor in 1938 was not significant.

The means for the sugar-beet varieties refer to the period July–October, omitting the later observations when N A R was very low and subject to large errors. In 1937 the variety Dobrovice N had on the average of all sowing

dates a significantly lower mean N A R than the other two varieties. There is an indication that the difference between Dobrovica N and Marsters decreased with later sowing, while the difference between Dobrovica and Kleinwanzleben E was little affected. The results for 1938 agree with those of 1937 in showing that Dobrovica N had a lower N A R than the other two varieties, but give no useful information on the effect of time of sowing, because the interval between the two sowing dates was very short, and no data were obtained on Marsters for the second date.

Commercial strains of sugar-beet fall into three groups, commonly distinguished as E, N, and Z strains. The Z strains, of which Marsters (derived from Hilleshög) is an example, were bred for the highest sugar content in the root. The E strains, such as Kleinwanzleben E, were bred for the highest yield, combined with a reasonable sugar content. They have larger leaves and a higher water content than the Z types. The N strains represent a compromise between high yield and high sugar content. Dobrovica N belongs to this group, but it is more like Kleinwanzleben E than Marsters and has even larger leaves than Kleinwanzleben E (see Table VIII).

The results for 1937 suggest that the strains with the larger and more succulent leaves, such as Kleinwanzleben E and Dobrovica N, differ from the Z strains such as Marsters in that their N A R is more greatly affected by time of sowing; while the N A R of the E and N strains was less than that of the Z strains when sown early, it differed little from or even exceeded that of the Z strains when sown late. The evidence for this is slender, but some support for it is given by the results of Boonstra (1939), who compared the growth of two strains, A and Z, of the variety Kuhn. A was a high-yielding strain with larger leaves and higher water content than Z, a high sugar-content strain. Boonstra found that A had a lower N A R than Z, and the difference was greater in 1934 when the date of sowing was April 20 than in 1935 when the date of sowing was May 10. However, the comparison between years is not a good one, for in the 1934 experiment the beet were grown in the field and in 1935 in pots. Further work, comparing E and Z strains of the same variety, is desirable to test this apparent difference between strains in response to time of sowing.

The 1937 data also provide some evidence that strains of similar type but of different varieties may differ in N A R; the N A R of Kleinwanzleben E was greater than that of Dobrovica N, and this difference varied little with time of sowing. In this connexion it would be of interest to determine whether a high N A R is characteristic of other strains of the Kleinwanzleben variety, by comparing Kleinwanzleben Z with other Z types such as Marsters.

The observations on potatoes in 1938 were not sufficiently precise, especially at the later samplings, to provide accurate information on the N A R of individual varieties. On the average of all sampling intervals, in the 12 weeks from mid-July to the beginning of October, there was no significant variation in N A R between varieties, but, as Table IV shows, such differences did occur in the earlier stages of growth. During the period July 14–August 25

up to about the time of maximum leaf area, the mean N A R of Ally was significantly greater, and that of British Queen less, than the means for the other varieties. In the subsequent period between August 25 and October 6, the errors became much greater and no significant differences between varieties were detectable. The results suggest that the high N A R of Ally was not maintained in this period; but the N A R of British Queen continued to be lower than that of the other varieties.

The effect of time of sowing

The average effects of time of sowing on N A R, for the two experiments in which sowing date was varied, are shown in Table V.

TABLE V

Regression Coefficients of Net Assimilation Rate on Time of Sowing in Weeks. (Mean of all Sampling Intervals from July 10 to October 30, 1934 and from July 5 to October 25, 1937)

1934		1937	
Sugar-beet:		Sugar-beet:	
Kleinwanzleben E	0.013 ± 0.0093	Kleinwanzleben E	0.018
Mangold:		Dobrovice N	0.020
Yellow Globe	0.011 ± 0.0064	Marsters	-0.002
Mean	0.012 ± 0.0077	Mean	0.012 ± 0.0049
Mean date of sowing	May 14	Mean date of sowing	June 1
Mean N A R	0.496	Mean N A R	0.481

The regression coefficients measure the change in mean N A R for the period July to October produced by 1 week's delay in sowing. In the 1934 experiment on sugar-beet and mangold no significant effect of time of sowing was detectable, but in 1937 two of the three varieties of sugar-beet showed a significant increase of N A R with later sowing, while the N A R of the third variety was apparently independent of sowing date. This difference between varieties in response to sowing date has already been discussed, and is set out in detail in Table IV. The effect of time of sowing did not vary significantly between sampling intervals in either experiment, nor was there any indication of a steady change in its magnitude associated with the fall of N A R as the season advanced. This is a surprising result, and it is possible that seasonal variation in the response to sowing date did, in fact, occur but the experiments were not sufficiently accurate to detect it.

Although the regression coefficients for the 1934 experiment did not differ significantly from zero, the coefficients for sugar-beet and mangold were very similar, and their mean was identical with that for the three varieties of sugar-beet in 1937. The experiments agree in showing that 1 week's delay in sowing caused an average increase of about 2.5 per cent. in N A R over the period July–October, but the 1937 experiment also indicates that the effect of time of sowing was not of the same magnitude for all varieties of sugar-beet.

There are two possible explanations of this effect of time of sowing on N A R. It may arise merely from the difference in age of early and late-sown

plants, and if so it implies that, independently of external factors, N A R declines with increasing age. There is no direct evidence of this from the data, for any such time drift of N A R induced by change in internal factors would be indistinguishable from the downward trend which has been attributed to seasonal variation in external conditions (Fig. 1). It is noteworthy that the effect of a difference of 1 week in age between sowings (Table V) was small compared with the mean weekly rate of decrease of N A R between July and October (p. 52), and this indicates that if there is a decline of N A R with age, it makes only a small contribution to the seasonal time trend. This explanation assumes that there is an ontogenetic decline of N A R which is similar for all sowing dates, but another possibility is that the internal factors vary with the time of sowing in such a way that independently of their age the leaves of late-sown plants have a higher N A R than those of early sown plants in comparable conditions. This does not exclude the possibility that N A R also varies with age. It is known that later sowing of sugar-beet and mangold causes an increase in the size and a reduction in the thickness of the leaves (Watson and Baptiste, 1938), and increases their sugar content (Watson and Selman, 1938), and it is not improbable that these, and possibly other, morphological and metabolic effects are associated with an increase in N A R.

The experiments of 1938 provide one other comparison of sowing dates—that between autumn-sown and spring-sown wheat of the variety Victor. The observations on the autumn-sown crop before ear emergence overlap with those on the spring-sown crop soon after germination in only one short interval; at this time the N A R of the spring-sown crop was slightly greater than that of the autumn-sown crop but the difference was not significant:

Date of sowing.	Sampling interval.	N A R.
Nov. 10, 1937	June 7-20, 1938	0.48 ± 0.053
May 20, 1938	June 14-21, 1938	0.54 ± 0.075

Further evidence that the two crops did not differ greatly in N A R is given by the time trend of N A R of the spring-sown crop (Fig. 1) which was a smooth continuation of that of the autumn-sown crop.

The relation of Net Assimilation Rate to climatic factors

The steady drift of N A R between low winter values and high summer values has been attributed to seasonal change in climatic factors, but it is not possible to distinguish which factors are responsible, for those which are likely to affect N A R, viz. temperature, length of day, and light intensity, have similar seasonal cycles and their time trends are highly correlated. However, information on the effect of individual climatic factors on N A R can be sought by investigating the relation between deviations of N A R and of these factors from their smooth time trends. The data are not very suitable for this purpose, for short-period variations are smoothed out when means are taken over the intervals of a fortnight or more over which N A R was measured.

Data for three species have been examined. The 1933-4 experiment on wheat provided determinations of N A R for 15 intervals before ear emergence. The results for potatoes in 1937 and 1938 were combined to give estimates of N A R for 12 intervals. Similarly, the data for sugar-beet and mangold in all 6 seasons were combined, using means for sugar-beet and mangold in 1934, and together provided estimates of N A R for 47 intervals between mid-June and early November. The weather variates selected were mean daily maximum and mean daily minimum temperatures, and mean radiation per day. Mean maximum and mean minimum temperatures were taken as measures of day and night temperature respectively, to avoid the labour of computing average temperatures for the hours of daylight and of darkness. Mean daily radiation takes account of variations in both day-length and light intensity, but the deviations from a smooth trend are attributable to short-period variations in light intensity, for day-length varies steadily with time.

For each set of data partial regressions of N A R on these three climatic factors were computed, after eliminating for each season a linear time trend. Although the true time trends of the climatic factors, and probably also of N A R, are harmonic curves, they are fairly well represented by straight lines over the periods investigated, from December to June, and from July to November. The results are shown in Table VI.

TABLE VI
Regression Coefficients of Net Assimilation Rate on Temperature, and Mean Radiation per Day

	Regression coefficients of N A R on: Temperature °F. ($\times 10^4$)				Mean radiation, Joules per sq. cm. per day ($\times 10^6$).	Variance accounted for by regression on tempera- ture and radiation, per cent.
	Mean daily maximum.	Mean daily minimum.	Daily mean.	Mean daily range.		
Potatoes 1937, 1938	386 \pm 216	-598 \pm 258	-106 \pm 50	984 \pm 466	-10 \pm 237	30.6
Wheat 1933-4	69 \pm 111	22 \pm 118	<u>45 \pm 18</u>	46 \pm 226	141 \pm 227	46.5
Sugar-beet* 6 years	-334 \pm 168	370 \pm 172	18 \pm 36	-703 \pm 332	522 \pm 264	6.4

* Mean of sugar-beet and mangold, 1934; mangold, 1939.
Significant results are underlined.

The mean of the regression coefficients on mean maximum and mean minimum temperatures represents the regression coefficient on mean daily temperature, and the difference between the regression coefficients on mean maximum and mean minimum temperatures is the regression coefficient on mean daily range of temperature. These regression coefficients on daily mean temperature and mean daily range are also given in Table VI; in computing their standard errors account has to be taken of the high correlation between daily maximum and minimum temperatures.

None of the regression coefficients on mean radiation per day was significant, but there is an indication that for sugar-beet N A R increased with increasing radiation, independently of associated temperature changes.

The most surprising feature of Table VI is that the relation of N A R to temperature varied with the species, not only in magnitude but in sign. The results for potatoes show a positive relation of N A R with day temperature but a negative relation with night temperature. In consequence, N A R increased with increasing daily temperature range. As the regression coefficient on maximum temperature was numerically less than that on minimum temperature, N A R was negatively related to daily mean temperature. None of the regression coefficients reached the 5 per cent. level of significance, but all except that for mean maximum temperature were nearly approaching significance. These results agree with those found for barley by Gregory (1926), and the magnitudes of the regression coefficients were very similar. Gregory's interpretation of the negative relation with night temperature is that high night temperatures increase respiration loss and so reduce N A R, while the positive relation with day temperature represents the effect of temperature on the rate of photosynthesis.

The regression coefficients on temperature for wheat were all numerically much smaller than those for potatoes. The only significant regression coefficient was that on daily mean temperature, which, unlike that for potatoes, was positive. There was no indication of an adverse effect of high night temperature, but as the regression coefficient on minimum temperature had a high standard error, it is possible that its true value was negative. The relation of N A R to day and night temperatures for wheat may, therefore, have been of the same type as that for potatoes, with the difference that night temperature had a relatively smaller effect.

The regression coefficients of N A R on temperature for sugar-beet were of opposite sign to those for potatoes. Increasing day temperature apparently reduced N A R, while increasing night temperature significantly increased it. Consequently, N A R was independent of daily mean temperature but decreased with increase in the daily range. This result is difficult to understand, but there seems to be no doubt of its reality; regression coefficients computed separately for each of the four years 1934, 1937, 1938, and 1939 gave values consistently of the same sign and of similar magnitude to those shown in Table VI, derived from the combined results of all six seasons, though they were significant in only one year. It seems unlikely that the relationships shown by the regression coefficients on temperature represent direct effects of temperature on N A R; a possible alternative explanation is that they show indirectly a relation between N A R and the water content of the leaf. During dry sunny periods in the summer months the leaves of field crops of sugar-beet frequently show severe wilting, often starting early in the day, which might be expected to reduce photosynthesis by stomatal closure. The daily range of temperature tends to be smaller in cloudy and rainy periods than when the sky is clear, and the inverse relation between

daily temperature range and N A R might be interpreted as showing that N A R tends to be high when conditions favour the maintenance of turgidity in the leaf, and low when conditions favour wilting. An attempt was made to test this hypothesis by computing the partial regression coefficient of N A R on mean water content of the leaves, using as an estimate of the latter the mean of the water contents determined at the sampling times at the beginning and end of each interval over which N A R was determined. Unfortunately this is unlikely to be a good estimate of mean water content over the interval, owing to the rapid and wide variation which may occur within short periods. The data for 1934, 1937, and 1938 were combined for this purpose. The regression coefficient of N A R on water content in gm. per 100 gm. dry matter had a value of $409 \pm 716 \times 10^{-6}$; though positive in accordance with expectation it was far from significant. The introduction of the water-content term into the regression equation did not change the sign of the regression coefficients on maximum and minimum temperatures, and only slightly reduced their magnitude. Thus there is no evidence that the regression coefficients of N A R on temperature represent effects of varying water content, but this is possibly due to the inadequacy of the data on water content. The elucidation of the temperature relationships requires more accurate estimates of N A R preferably over shorter intervals of time, but the results show that the relation of N A R to day and night temperatures found by Gregory, for barley, and confirmed for potatoes, apparently does not hold for all crops in field conditions.

The fractions of the total variance between sampling intervals of the deviations of N A R from a smooth trend accounted for by the regressions on temperature and radiation (Table VI) were small compared with the value of 80 per cent. found by Gregory in his experiments on barley. The difference is probably accounted for by the much higher sampling errors of the field material compared with plants grown in pot-culture, though it is possible that other factors which have not been taken into account may play a more important part in field conditions than in pot-cultures.

Part II. Leaf Area

Variation within and between years

The changes in leaf area per metre-length of drill row during the growth of the wheat and barley crops are shown in Fig. 3A. In the winter months the leaf area of autumn-sown wheat was very small, and increased only slowly. From March onwards the expansion of leaf area became more rapid and continued throughout April and May to a maximum in early June. This was followed immediately by a steady fall to zero at the end of July. The leaf area of spring-sown barley varied similarly to that of wheat, but the sequence of changes occurred about one month later and the maximum leaf area was reached at the end of June.

The relation between the variation in leaf area and other morphological changes is shown by comparison with the graphs of shoot number and shoot

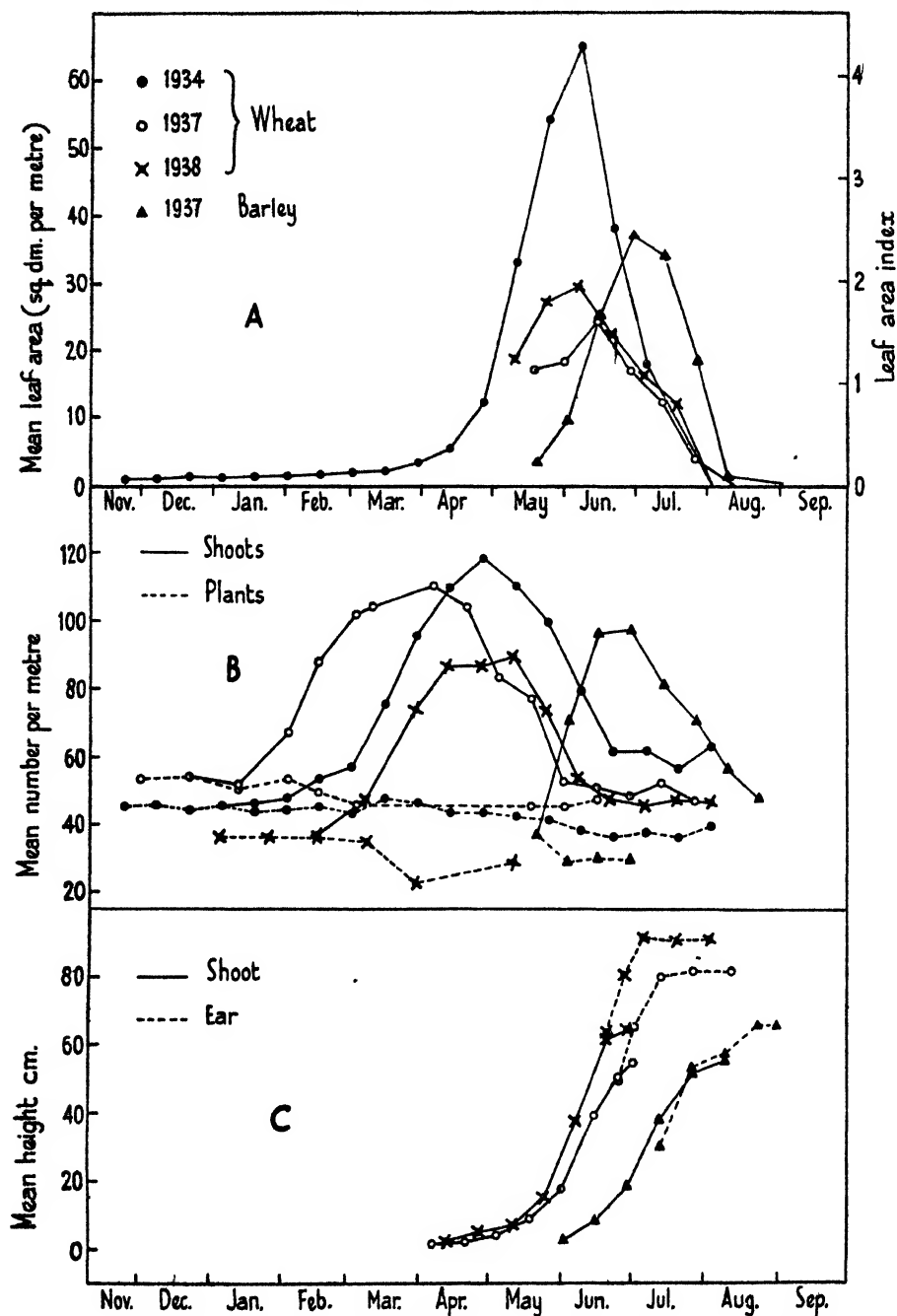


FIG. 3. Variation with time in the leaf area, shoot number, and plant number per metre of drill row, and in shoot and ear height of wheat and barley crops. (Shoot height was measured to the auricle of the highest leaf; ear height was measured to the tip of the ear, excluding the awns of barley.)

and ear height in Fig. 3B and C. In wheat, the rapid increase of leaf area began at about the time of maximum shoot number, so that most of the phase of increasing leaf area occurred when shoot number was declining. In barley, on the other hand, leaf area and shoot number increased together and attained their maxima nearly simultaneously. The leaf area of wheat was maximal at about the time when the death of tillers ceased and shoot number had fallen to the level which was maintained until harvest. The subsequent fall of leaf area resulted from the senescence and death of the leaves on the ear-bearing shoots in succession from the base upwards, but in barley the fall was also attributable in part to the death of shoots.

Maximum leaf area in both wheat and barley occurred during the phase of rapid shoot elongation, at the time when the shoots had reached about half their final height. At ear emergence, shown in Fig. 3C by the divergence of the graphs of ear height and of shoot height, leaf area had fallen to about two-thirds of its maximal value.

In all the crops the number of plants per metre (Fig. 3B) decreased slightly during growth, but the fall was too slight to play an appreciable part in determining the leaf area of the crop.

Comparison of the results for the three wheat crops in Fig. 3 shows that, in the period from May onwards, leaf area in different years increased with increasing shoot number, but the greater leaf area per metre in 1934 than in 1937 and 1938 was due partly to a greater leaf area per shoot as well as to a higher shoot number. No counts were made of leaf number per shoot or per metre, but the higher leaf area per shoot in the 1934 crop probably indicates that the mean leaf size was increased. There was no simple relation between maximum shoot number and leaf area at subsequent stages. The 1937 crop had a maximum shoot number considerably greater than that of the 1938 crop, and nearly as great as the 1934 crop, but it had the smallest leaf area per metre throughout. Although tillering began, and reached its maximum, earlier in 1937 than in the other two years, maximum leaf area was attained slightly later in 1937 than in 1934 and 1938.

Fig. 4A shows the changes with time in the leaf area per plant of the sugar-beet and potato crops. The leaf area per plant of sugar-beet increased rapidly during the period June–August, but subsequently the increase continued only slowly or there was a slight fall. The shape of the leaf-area curve has previously been shown to depend on the date of sowing. Watson and Baptiste (1938) found that the leaf area per plant of sugar-beet sown in early April ceased to increase after the beginning of August, while that of sowings made in late May or June continued to increase until November. Consequently, late sowing reduced the leaf area per plant in the early stages of growth, but from September onwards the effect was reversed and the leaf area per plant increased with later sowing. A difference of this nature is apparent in Fig. 4A between the graphs for 1942 and 1943, when the crop was sown in mid-April, and those for 1934 and 1938, which are each the mean of several sowings, with mean sowing dates of May 14 and 15 respectively. But the differences between years

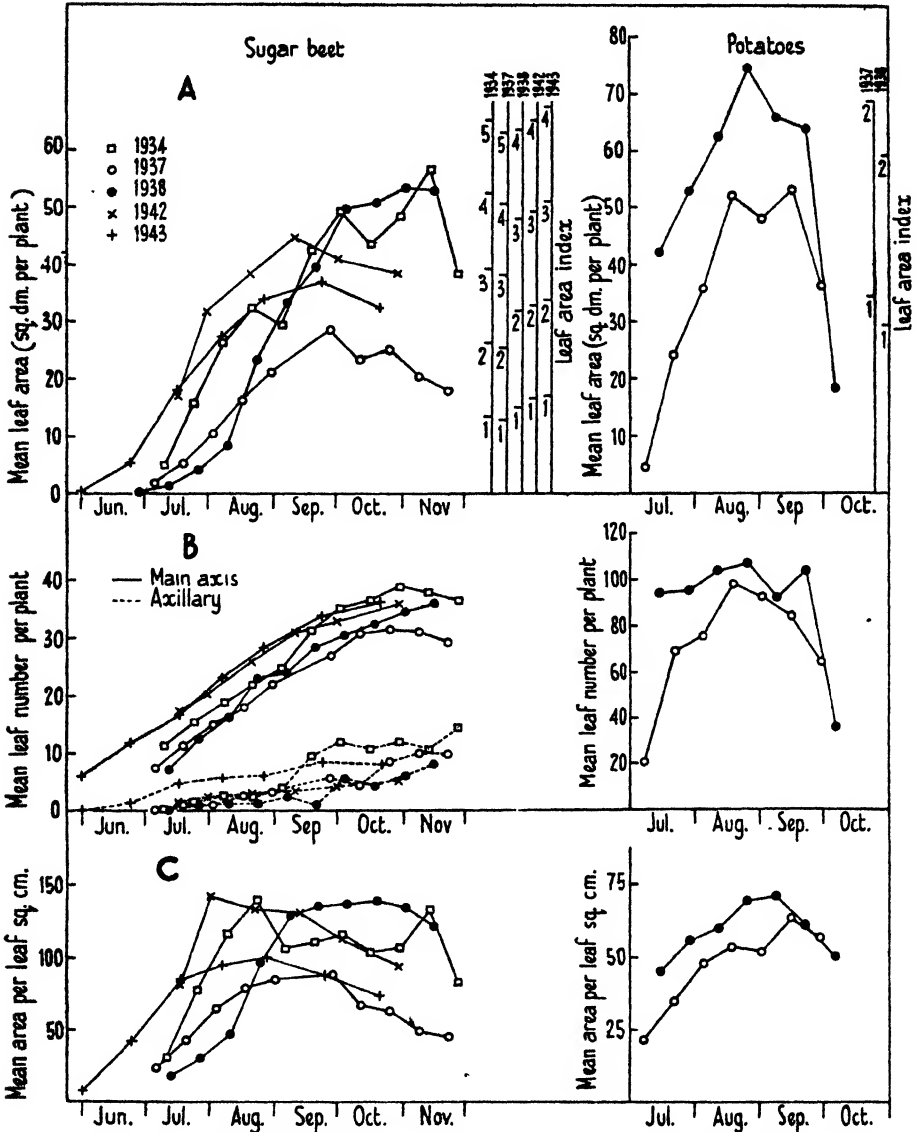


FIG. 4. Changes with time in the leaf area per plant, number of leaves per plant, and mean area per leaf of sugar-beet and potato crops.

cannot be accounted for entirely by variation of sowing date, for the curve of leaf area per plant for 1937, when the mean date of sowing was May 23, was more like those of the early sown crops of 1942 and 1943, than the late-sown crops of 1934 and 1938. However, the results for successive sowings in 1937 (Table VII) showed the usual response of leaf area per plant to time of sowing.

TABLE VII

Effect of Date of Sowing on Leaf Area and Leaf Number of Sugar-beet, 1937
Mean of Three Varieties

Date of sowing.	Mean leaf area (sq. dm.) per plant.		Mean leaf number per plant.*		Mean area (sq. cm.) per leaf.	
	A	B	A	B	A	B
Apr. 30. .	12	17	20	40	56	47
May 25. .	12	24	16	37	65	72
June 12. .	7	28	11	35	41	85
S.E. . .	0.5	1.2	0.3	1.7	2.2	3.9

* Including leaves produced from axillary buds.
Periods averaged. A, July 5–Aug. 30; B, Sept. 27–Nov. 22.

Table VII also shows that the increase of leaf area per plant with later sowing, which occurred from September onwards, was attributable to an increase in leaf size (mean area per leaf) and not in leaf number, although the difference in leaf number between sowings diminished with time. This is in agreement with previous results (loc. cit.).

The leaf number per plant of sugar-beet (Fig. 4B) increased steadily until October; in 2 years this was followed by a slight fall in November. The mean area per leaf (Fig. 4C) also increased rapidly during June and July, and longer in the later-sown crops, but from August onwards it varied irregularly, usually tending to fall, so that the increase in leaf area per plant after August was determined wholly by the increase in leaf number.

The variation between years in leaf number per plant at comparable dates was small, and was mainly attributable to differences in sowing date. Thus the mean numbers of leaves per plant produced from the apical bud (main axis, Fig. 4B) were almost identical in the two seasons, 1942 and 1943, when the crops were sown in April. Similarly, the 1937 and 1938 crops, which had a later mean date of sowing, had almost equal numbers of leaves per plant, but in 1934 leaf number was consistently higher. As there was a greater development of leaves from axillary buds in 1943 and 1937 than in 1942 and 1938, the greater leaf area per plant in the two latter years was entirely the result of a greater mean area per leaf. However, the high leaf area per plant in 1934, compared with 1937, was partly attributable to high leaf number, but the difference in leaf number between the two seasons was relatively smaller than that in mean area per leaf. It seems justifiable to conclude that variation in leaf size was a more important cause of differences in leaf area per plant in different years than variation in leaf number.

The leaf area per plant of potatoes (Fig. 4A) increased during July and early August to a maximum, which was not so sharply defined as in the cereal crops, and then fell rapidly in late September, as the haulms died off. Leaf number per plant and mean area per leaf showed similar time changes. Increases in both leaf number and mean area per leaf contributed to produce the greater leaf area per plant in 1938 than in 1937. The 1937 crop was planted about

one month later than the 1938 crop, and this accounts for the large differences between the crops in leaf area and leaf number during July and early August. At the time of maximal leaf area per plant the crops showed a relatively greater difference in mean area per leaf than in leaf number.

A comparison of the leaf area per plant of different species is of little interest, for these must be highly dependent on the spacing of the plants, which varied in accordance with normal agricultural practice. The measure of leaf area which is relevant to the comparison of agricultural yields, that is, of the weights of different crops produced per unit area of land, is the leaf area per unit area of land, which it is proposed to call the Leaf Area Index (L A I). Scales of L A I are given at the right-hand sides of Figs. 3 and 4. A value of 2 for L A I, for example, indicates that there were 2 acres of leaf surface on an acre of crop. The differences between crops in L A I will be discussed later; it is sufficient to note here that L A I at the time of maximum leaf area was of the same order of magnitude for the cereal and sugar-beet crops, ranging from 2 to 4, and slightly less, 1.5 to 2.5, for potatoes.

Varietal differences

The differences between varieties in leaf area and in leaf number were qualitatively the same throughout growth, though their magnitudes tended to increase as leaf area and leaf number increased. They can therefore be represented adequately by the differences between means taken over a long period so as to reduce sampling errors (Table VIII).

The means for wheat and sugar-beet refer to periods when leaf number and leaf area were near their maximal values; those for potatoes cover the whole period of observations.

In both 1937 and 1938 Yeoman had a slightly smaller leaf area per metre of drill row than the other two wheat varieties. Although, as is well known, Yeoman tillers more freely than other common wheat varieties, its high shoot number did not completely compensate for its small leaf area per shoot. No counts of leaf number per shoot were made, but it was obvious from inspection that Yeoman had smaller leaves than the other varieties, and this was probably sufficient to account for the smaller leaf area per shoot.

Two of the three varieties of sugar-beet, Kleinwanzleben E and Dobrovice N, had almost identical mean leaf areas per plant. Dobrovice N had rather fewer but larger leaves than Kleinwanzleben E. The mean leaf area per plant of Marsters was about 70 per cent. of those of the other varieties, in both 1937 and 1938. Marsters had more leaves, especially on axillary buds, but its leaves were, on the average, only about half the size of those of Kleinwanzleben E and Dobrovice N. The smaller mean area per leaf of Marsters was partly the result of the greater number of axillary leaves, for in all three varieties these leaves were much smaller when fully expanded than leaves produced from the apical bud.

The differences in leaf area per plant, and in leaf size and number between the Z type strain, Marsters, and the E and N strains, agreed with those

described by Boonstra (1937) between Z and A strains of the variety Kuhn. Differences of the same nature were also found by Watson and Baptiste (1938) between Kleinwanzleben E sugar-beet and mangold. Evidently, as dry-matter and sugar contents increase over the wide range of agricultural varieties of *Beta vulgaris*, from the mangold at one extreme to Z-type strains of sugar-beet at the other, leaf size tends to fall and leaf number to rise, and this suggests that meristematic activity and leaf growth are causally related to dry-matter content and sugar content.

TABLE VIII
Leaf Area and Leaf Number of Different Varieties

WHEAT									
1937 (May 18-June 28)					1938 (May 9-June 20)				
Variety	Square-head's				S.E.	Square-head's			
	Master.	Yeoman.	Victor.			Master.	Yeoman.	Victor.	S.E.
Leaf area (sq. dm. per metre)	19	17	22	0.8		25	23	25	0.4
Shoots per metre	53	65	54	1.8		64	78	57	1.2
Leaf area (sq. cm. per shoot)	36	27	41	1.0		43	32	46	0.4

SUGAR-BEET									
1937* (Sept. 27-Nov. 22)					1938† (Sept. 20-Nov. 15)				
Variety	Dobro-				S.E.	Dobro-			
	Klein. E.	vice N.	Marsters.			Klein. E.	vice N.	Marsters.	S.E.
Leaf area (sq. dm. per plant)	25	25	18	1.2		49	55	35	2.4
Leaf number per plant	Main axis	29	28	32	0.8	33	32	35	1.6
	Axillary	6	4	12	1.5	3	1	10	1.8
	Total	35	31	44	1.7	36	33	45	3.1
Mean area (sq. cm. per leaf)	78	83	43	3.9		139	169	84	1.9

POTATOES							
1938 (July 14-Oct. 6).							
Variety	Great		Majestic.	Arran		British	S.E.
	Ally.	Scot.		Banner.	Queen.		
Leaf area (sq. dm. per plant)	48	48	53	62	58		2.8
Leaf number per plant	85	76	107	84	100		4.6
Mean area (sq. cm. per leaf)	55	66	48	72	57		0.9

* Mean of 3 sowing dates.

† First sowing, 13 May.

The higher leaf number per plant of the Z type of Kuhn compared with the A type, and of Kleinwanzleben E sugar-beet compared with mangold (loc. cit.), was partly the result of a lower death-rate of the leaves, as well as of an increased rate of leaf production. In both cases the leaves of the varieties with the higher dry-matter content and sugar content had a longer life. No information on the longevity of leaves was obtained in the experiments described in this paper, but it is possible that differences in longevity played some part in determining varietal differences in leaf number and leaf area per plant in the other crops, as well as in sugar-beet. Boonstra (1929 a) has shown,

for example, that there are differences between varieties of oats in the length of life of their leaves.

Of the five varieties of potatoes compared in 1938, Arran Banner and British Queen had a significantly greater mean leaf area per plant than Ally and Great Scot, with Majestic occupying an intermediate position. As with wheat and sugar-beet, there was a tendency for high leaf number per plant to be associated with small leaves, so that both leaf number per plant and mean area per leaf showed greater variation between varieties than leaf area per plant.

The general statement made above that varietal differences were qualitatively the same throughout growth was not strictly true of potatoes. Leaf area decreased more rapidly during September in British Queen than in the other varieties. Until the end of August, British Queen had the largest leaf area per plant, but by the end of October it had fallen to the lowest position. Similarly Great Scot showed a more rapid fall of leaf area than Ally, Arran Banner, and Majestic. Consequently, if the period from the end of August onwards is omitted in computing mean leaf area per plant (Table IX), the values for British Queen and Great Scot are greater relative to the means for the other varieties than when the means are taken over the whole period of observation (Table VIII). The change in the order of the varieties in respect of leaf area arose from the earlier maturation and death of the haulms of British Queen and Great Scot, and is in accordance with the usual classification of these varieties as second-earlies; the other three are classed as early main-crop varieties.

DISCUSSION

Heath and Gregory (1938) concluded from their survey of the data then available that there was little or no variation between species in N A R. The results of the present work do not confirm this; on the contrary, all the four species tested differed from each other. The dicotyledons, sugar-beet and potatoes, had higher N A Rs than the cereals, barley and wheat, and in the extreme case the N A R of sugar-beet was nearly twice that of wheat. There is evidence of a difference between barley and sugar-beet in the table given in Heath and Gregory's paper, but it is partly obscured by the circumstance that the values given for barley refer to the period May–July, when N A R is nearly maximal, while those for sugar-beet extend over a longer period into the late summer and autumn when seasonal weather conditions cause a rapid fall of N A R. Also, the mean values of N A R for barley given in the table are somewhat higher than those found for field crops of cereals in the present experiments. Gregory's data were obtained from pot-cultures and this may account for the difference, for in pot cultures the plants are not so closely crowded as in field conditions and are therefore less subject to competition for light and CO₂. This suggests that the contrast in N A R between root crops and cereals in the field may be determined partly by the wide difference between them in the spacing of the plants.

the Growth of Field Crops. I

Differences in N A R between varieties, of sugar-beet and potatoes but not of wheat, were also established. As might be expected, these were small compared with the inter-specific differences. They were independent of any effect of spacing.

Briggs, Kidd, and West (1920), Gregory (1926), and others have shown that N A R is subject to variation by short-term changes in climatic factors, especially temperature, and this was confirmed, though there was evidence that the relation of N A R to temperature varied in different species. Heath and Gregory (1938) have maintained that, apart from such short-period fluctuations, differences in the environment do not affect N A R, and have deduced that there must exist some measure of compensation by internal changes in the leaf, whereby plants are adapted to produce in all environments approximately the same quantity of dry matter per unit leaf area. If such compensation occurs, it is evidently insufficient to smooth out completely the effect of differences in the environment of a crop grown in the same locality in different years. For each species tested differences between years were found in the mean N A R for the same calendar period of two or more months. These may have been caused partly by differences between years in weather conditions, and partly by differences between fields in soil conditions. Gregory's results for barley in pot-culture show similar variation in N A R between years. The existence of a seasonal trend of N A R is also evidence against the view that compensation occurs for persistent differences in environmental factors. Heath and Gregory's hypothesis of the constancy of N A R in different environments was based on contrasts which all involved differences of species as well as of environments. It is therefore possible that N A R was apparently constant, not because it was independent of both species and environment, but because both had effects which were opposite in the conditions compared. That is to say, in the data given in Heath and Gregory's table the species of higher N A R may be associated with the less favourable environments. It has already been shown that this is the explanation of the approximate equality of the values of mean N A R for barley and sugar-beet.

Although the downward trend of N A R from high summer values to low values in the late autumn was shown by the results of Watson and Baptiste (1938) and Boonstra (1939), the complete cycle of seasonal fluctuation has apparently not been demonstrated previously. The great difference in the rate of growth of field crops in summer and in winter is sufficient to suggest that seasonal variation of N A R occurs, and possibly it has been thought too obvious to be worthy of comment. Nevertheless, the seasonal cycle is of great importance in relation to dry-matter accumulation in different species. There is no rigid proof that the seasonal cycle is induced by seasonal change in climatic conditions, but any other explanation seems very improbable.

Comparisons of crops sown on different dates suggest that the N A R of both sugar-beet and wheat tended to fall with advancing age, but the effect was small and scarcely detectable. Hence, the seasonal time-trend of N A R cannot be attributed to ontogenetic changes in internal factors, especially as

this would imply that the N A R of wheat increased with age, while that of sugar-beet decreased. The increase of N A R in sugar-beet during the early stages of growth up to the end of June also makes it difficult to attribute the subsequent fall to an age effect.

It appears, then, that N A R varies between species, between varieties of the same species, between years, between seasons of the year, and probably with age. This scarcely accords with Heath and Gregory's view that, excluding variation caused by short-term changes in climatic factors, 'the mean N A R in nature is no very variable quantity'. Nevertheless, it is true that variation of N A R is of minor importance in determining differences in yield between years and between varieties of the same species. Table IX (a) shows the total dry weight at harvest of crops of wheat, potatoes, and sugar-beet grown in different years, with the corresponding values of mean leaf area, and mean N A R, for the longest calendar period of observation common to all years.

TABLE IX
The Relation of Dry-matter Accumulation to Leaf Area and Net Assimilation Rate

(a) Comparison of Different Years.

	Wheat.			Potatoes.		Sugar-beet.				
	1937.	1938	1934.	1937.	1938.	1937.	1938.	1942.	1934.	1943.
Dry matter at harvest*	79	158	197	218	262	105	147	208	212	231
Mean leaf area†	19	24	48	36	54	16	26	35	32	30
Mean N A R‡	0.32	0.50	0.39	0.49	0.51	0.42	0.49	0.35	0.51	0.46

(b) Comparison of Varieties.

	Wheat, 1937.			Wheat, 1938.		
	Square-head's			Square-head's		
Variety	Yeoman.	Master.	Victor.	Yeoman.	Victor.	Master.
Dry matter at harvest (gm. per metre)	74	76	88	149	160	166
Mean leaf area (gm. per metre)§	17	19	22	23	24	25
Mean N A R§	0.32	0.33	0.33	0.49	0.53	0.48

	Potatoes, 1938.				Sugar-beet, 1937.		
	Ally.	Great Scot.	Majestic.	Arran Banner.	British Queen.	Dobrovice N.	Klein. E.
Variety							
Dry matter at harvest (gm. per plant)	231	256	263	268	294	99	100
Mean leaf area (sq. dm. per plant)§	50	53	56	62	68	14	18
Mean N A R§	0.28	0.35	0.37	0.31	0.24	0.44	0.36

* gm. per metre of drill row for wheat; gm. per plant for potatoes and sugar-beet. The values for sugar-beet are the weights at the end of October.

† sq. dm. per metre of drill row for wheat; sq. dm. per plant for potatoes and sugar-beet.

‡ Periods averaged: wheat, May-June; potatoes, July-Sept.; sugar-beet, July-Oct.

§ Periods averaged: wheat, May-June; potatoes, July-Aug.; sugar-beet, July-Nov.

Table IX (b) gives similar data for comparison of varieties of each crop within years. In calculating the values of mean leaf area and mean N A R for the potato varieties, the period after the end of August, when differences in the rate of maturation became apparent, was omitted. N A R was low at this period, so that differences in leaf area which developed then had little effect on total dry weight at harvest. The variation in mean leaf area between years

and between varieties was relatively much greater than the variation in mean N A R, so that throughout the table increase in dry-matter accumulation is associated with increasing leaf area, but there is no obvious correlation between dry-matter accumulation and mean N A R. Evidently variation of leaf area was a much more potent factor than variation in N A R in determining differences in dry-matter accumulation between years and between varieties.

It is more difficult to assess the relative importance of leaf area and N A R as factors determining differences between species in yield of dry matter, because the growth period varies in length and occurs at different seasons of the year in different species. The relevant data for such an assessment are given in Fig. 5, which shows the changes with time in N A R and L A I of wheat, barley, potatoes, and sugar-beet. The curves of N A R in the upper half of the figure were obtained by drawing smooth freehand curves through the points in Fig. 1, to eliminate the effects of short-period climatic changes and of sampling errors, and then averaging values for comparable dates on the curves for different years. The dotted parts of the curves are extrapolations over parts of the growth period not covered by the observations, namely, the period after ear emergence of wheat and barley and the early stages of growth of potatoes. The results for 1939 were excluded because the conditions of growth of the crops in that year differed greatly from those of the other years, and from normal agricultural practice. Thus, the curves are smoothed averages of the results of 5 years of sugar-beet crops, 2 years of potatoes, only 1 year of barley, and 4 years of wheat (except that data for the period from sowing to early May were obtained in only one year). They show clearly the fluctuations of N A R between a summer maximum and a winter minimum, and the differences between species which have been described previously.

The lower half of Fig. 5 shows smooth curves representing the average changes with time in L A I for each crop, derived from the graphs of leaf area in Figs. 3 and 4, in a similar manner to the N A R curves. The curves of L A I for the four crops are of similar shape and differ mainly in their position on the time axis. Other obvious differences are the lower maxima in barley and potatoes than in wheat and sugar-beet, and the less abrupt fall from the maximum in sugar-beet than in the others.

Now, the measure of leaf area which is relevant to comparisons of total dry-matter accumulation in different species is clearly the integral of L A I over the whole growth period, for this takes account of both the magnitude of leaf area and its persistence in time. As it has the dimension of time, it can conveniently be called Leaf Area Duration (L A D). (This function is a measure of the ability of the plant to produce and maintain leaf area, and hence of its whole opportunity for assimilation; in conditions of constant N A R, dry-matter accumulation would be proportional to L A D. The values of L A D for each crop, measured from the curves in Fig. 5 by means of a planimeter, are shown in Table X, in comparison with the mean yield of dry matter at harvest.

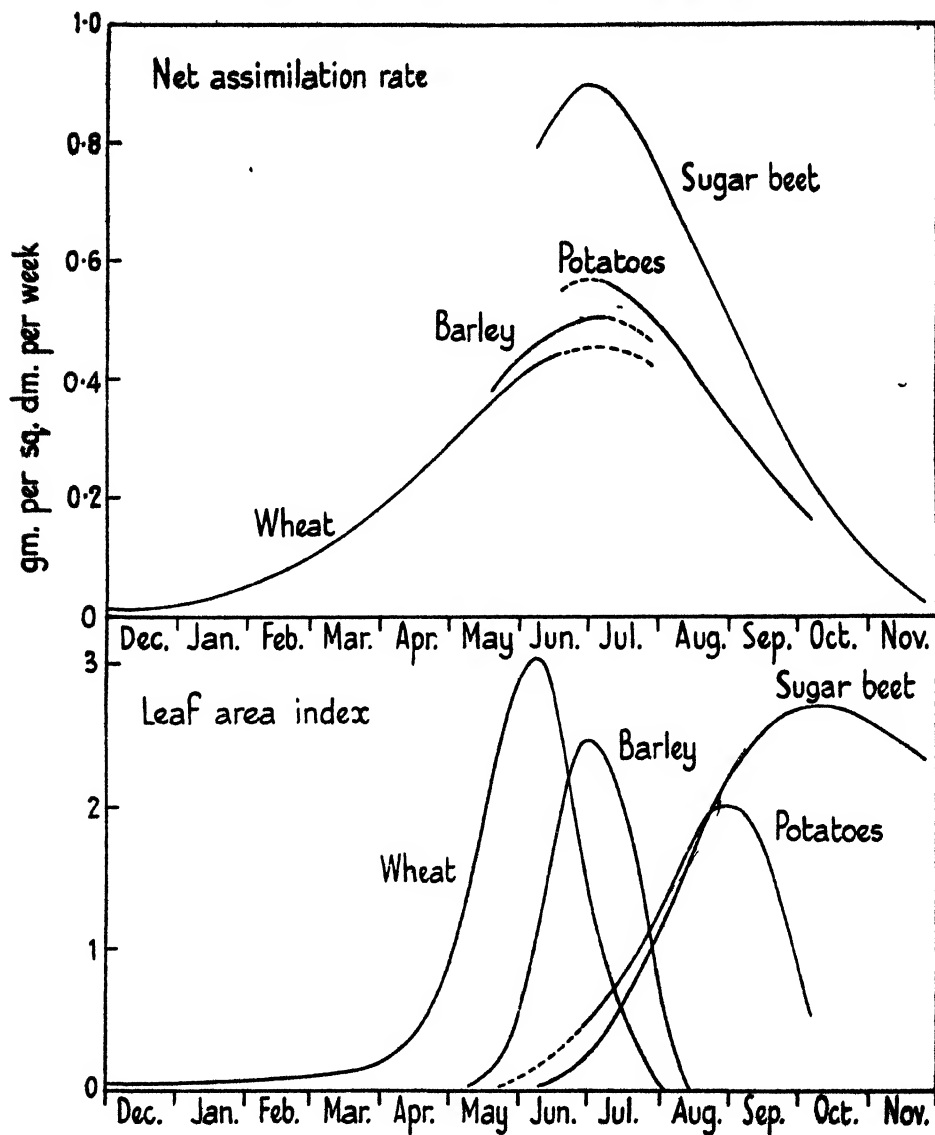


FIG. 5. Smoothed curves showing the average change with time in net assimilation rate and leaf area index of different species.

The dry weight of the different crops at harvest increased steadily with increase in L A D, and the ratio of dry-weight yield to L A D was almost constant. This ratio is shown in the third column of Table X. It is a measure of mean N A R, in cwt. per acre of leaf surface per week, the value of N A R at any time being weighted proportionally to the magnitude of L A I at the time. The fourth column of Table X gives the weighted mean N A R after

conversion to the usual units, gm. per sq. dm. per week. The values of mean N A R for barley and wheat are slightly higher than those for potatoes and sugar-beet, but if allowance were made for the fact that part of the dry matter accumulated, probably about 10–15 per cent., is attributable to photosynthesis in the ear, the differences between crops would be reduced.

TABLE X

The Relation of Dry Weight at Harvest to Leaf Area Duration and to Net Assimilation Rate in Different Crops

	Mean dry matter at harvest (cwt. per acre).	Mean L A D, weeks.	Weighted mean N A R. Cwt. per acre per week.	Gm. per sq. dm. per week.
Barley . . .	58	17	3·4	0·43
Potatoes . .	61	21	2·9	0·36
Wheat . . .	76	25	3·0	0·38
Sugar-beet .	96*	33	2·9	0·36

* At the end of October.

The conclusion to be drawn from Table X is that the differences in dry-matter accumulation between species, like those between years and between varieties of the same crop, can be accounted for almost completely by differences in leaf area. The explanation of the constancy of the weighted mean N A R is easily seen from Fig. 5. Although sugar-beet and potatoes had a greater N A R than the cereals, at comparable times in the seasonal cycle, this difference was offset by the fact that the leaf area of the cereals was greatest in May and June near to the time when N A R was also maximal, whereas the leaf areas of potatoes and sugar-beet were small at the time of the seasonal peak in N A R, and reached their highest values later in the year when N A R had fallen to a much lower level. Similarly, the slight superiority of potatoes over sugar-beet in L A I during June and July, when N A R was high, was sufficient to counterbalance the much greater deficiency of potatoes compared with sugar-beet in L A I in September and October, when N A R was low.

It should be noted that the data set out in Fig. 5 and Table X refer to a very small number of crops all grown in one locality and cannot be taken as representative of the average differences between crops growing in normal agricultural conditions in this country. It is probable, for example, that if more extensive data were available, the order of the four crops in respect of dry-matter production shown in Table X would be changed, and that potatoes would occupy a higher position.

It is obvious from a consideration of the changes with time in N A R and in L A I set out in Fig. 5 that a plant will operate with maximum efficiency as a producer of dry matter if its greatest leaf area is developed at the time when seasonal conditions are most favourable for photosynthesis. In this respect barley was the most efficient of the four species investigated, for its maximum L A I coincided with the midsummer peak of N A R. Wheat was

somewhat less efficient, though consideration of its leaf area alone probably leads to an underestimation of its efficiency, for in July the ears supplement the leaves as organs of photosynthesis. Potatoes and sugar-beet were, by comparison with the cereals, very inefficient; they produced their greatest leaf area too late to take advantage of the high N A R of the summer months.

From the practical point of view it is of interest to consider whether the change of L A I with time can be altered in relation to the seasonal trend of N A R, by change of cultural methods, so as to improve the efficiency of crops as producers of dry matter. Fig. 5 suggests that, for potatoes and sugar-beet, the object should be to induce a greater development of leaf surface during the summer months, and the obvious way of doing this is by earlier planting or sowing. For sugar-beet it has been shown (Watson and Baptiste, 1938) that earlier sowing has this desired effect on leaf area in June and July, but in later months the effect is reversed, and this tends to reduce the beneficial effect on yield. Moreover, a limit to the earliness of sowing or planting is set by the physical condition of the soil as well as by the possibility that very early sowing may cause bolting in sugar-beet or lead to damage by frost to potatoes. Fig. 5 also suggests that the dry-matter yield of wheat might be increased if the development of leaf area were delayed. Spring sowing, instead of autumn sowing, may have such an effect, but it evidently has other undesirable effects, causing a reduction of L A D, for it is well known that spring wheat produces a lower yield than autumn wheat. An alternative method of delaying the production of leaf area might be by the selection of late-maturing varieties, but against any benefit to be derived in this way must be set the inconvenience and dangers of a late harvest.

SUMMARY

Determinations of net assimilation rate (N A R) and of leaf area per plant or per metre of drill row were made at intervals throughout the growth of field-crops of wheat, barley, potatoes, mangolds, and sugar-beet at Rothamsted. Data are presented from 15 experiments in 6 years. The experiments included comparisons of varieties of the same crop, and of sowings made on different dates.

Net assimilation rate showed similar variation with time in all years, rising during the spring months from very low values in winter to a maximum at midsummer, and subsequently falling during the late summer and autumn. This time trend is attributed to seasonal change in climatic factors. Deviations from a smooth time trend were found to be correlated with temperature deviations. The relation of N A R to temperature varied in different crops; for example, that of potatoes increased, while that of sugar-beet decreased, with increase in the mean daily temperature range. No significant correlation between N A R and mean daily radiation was detected.

Net assimilation rate measured for the same species over comparable calendar periods was found to vary from year to year. This annual variation may have been due partly to differences between years in climatic conditions, and

partly to differences between fields in soil conditions. The existence of a seasonal time trend and of annual variation in N A R is evidence against the hypothesis that, apart from short-period fluctuations, N A R is independent of environmental conditions.

All the species tested were found to differ in N A R, when compared over the same calendar period in the same year. The rate increased in the order: wheat, barley, potatoes, mangolds, sugar-beet. At the extremes the N A R of sugar-beet was about twice that of wheat. Small but significant differences of N A R were also found between varieties of sugar-beet and potatoes, but not of wheat.

The net assimilation rate of sugar-beet was slightly increased by later sowing. This may imply that N A R declines with age, independently of change in external factors, but other explanations are possible. Autumn-sown and spring-sown wheat of the same variety differed little in N A R. These results show that variation of N A R with age plays little if any part in determining seasonal variation.

The leaf area per metre of drill row of wheat began to increase rapidly at about the time of maximum shoot number, reached its maximum in early June during the phase of shoot elongation when the shoots had attained about half their final height, and then decreased to zero at the end of July. The sequence of changes in the leaf area of barley was similar, but occurred later in the year; in contrast with wheat, leaf area and shoot number reached their maxima at about the same date, at the end of June. Potatoes attained their maximum leaf area per plant in August, and like the cereals showed a rapid decrease of leaf area in the later stages of growth. The change with time in leaf area per plant of sugar-beet depended on the date of sowing. Early sowings produced their maximum leaf area in September, and subsequently there was a slow fall, but the leaf area of late sowings continued to increase into November.

Differences between years and between varieties in leaf area per plant of sugar-beet were mainly due to variation in leaf size and not in leaf number per plant. This was probably also true of wheat, but in potatoes, variation of leaf number also contributed to determine the differences between varieties in leaf area per plant.

Net assimilation rate was much less variable between years and between varieties of the same species than leaf area. Consequently the differences between years and varieties in yield of dry matter reflected mainly the differences in leaf area, and showed no close relation to the much smaller differences in N A R. Similarly, differences between species in yield of dry matter were accounted for almost completely by differences in leaf area, in spite of the wide variation in N A R between species. The reason is that in different species the production of leaf area occurred at different stages of the seasonal cycle of N A R. In general, variation in leaf area was the main factor determining differences in yield; variation in N A R was of minor importance.

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Studies on the Morphology and Parasitism of *Hemileia* Species on Rubiaceae in Mysore

BY

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With fifteen Figures in the Text

THE genus *Hemileia*, first described by Berkeley and Broome to accommodate the rust fungus on coffee leaves in Ceylon and based on the material collected by Dr. Thwaites, includes several species of which the coffee leaf rust *Hemileia vastatrix* Berk. & Br. has been the object of many detailed investigations. Species of *Hemileia* are known to parasitize the members of Rubiaceae, Verbenaceae, Asclepiadaceae, Apocynaceae, Capparidaceae, and Araliaceae among dicotyledons and Dioscoreaceae and Orchidaceae among the monocotyledons. Recently Cummins (1943) has described another species on the Hypericaceae, and Smith (1934) doubtfully assigns to this genus a rust species on *Pimenta officinalis* collected in Jamaica. All the species of *Hemileia* so far known are hemi-forms, the pycnial and aecial stages being so far unknown. In most of the cases the identity of the rust is based on the characteristic shape and sculpturing of the urediospores rather than the telia, which are generally present only for a short period. In the search for the aecial stages for the genus *Hemileia* only one instance can be quoted where a definite claim of the discovery of the aecial stage has been made. Stevens (1928) described what he considered to be the aecial stage for the rust *Hemileia Wrightiae* Racib. in the Philippines. The aecia were stated to be uredinoid, the sori appearing bright reddish in colour. The aeciospores, according to Stevens, resemble the urediospores in all respects except that they have smaller aculeate processes and that on germination they develop unbranched germ-tubes unlike the urediospores. No pycnia were seen to accompany such uredinoid aecia.

Hemileia Wrightiae has been collected in abundance round about Bangalore, and sporidial inoculation experiments carried out by one of the writers (Thirumalachar, 1946) have failed to confirm the autoecious nature of the rust. Slight differences in the shades of colour of the spores are so often observed that no special significance can be attached to that. It seems therefore likely that the so-called aecial stage described by Stevens was an uredo stage.

Unconnected aecial stages occur on the same hosts along with *Hemileia* species in *Hemileia Woodii* Kalch. & Cooke on *Vangueria spinosa*, and *Hemileia*

Canthii Berk. & Br. on *Plectronia parviflora*. *Aecidium nobile* Syd. was recorded by Sydow and Butler (1906) as being parasitic on the leaves of *Coffea arabica* in Mysore. The species was based on the collections made by Butler in Barguai estate in Mysore and has never since been collected. However, a re-examination of the type material deposited in the Herb. Crypt. Ind. Orient., New Delhi, by Mundkur and Thirumalachar (1946) has revealed that *Aecidium nobile* Syd. is synonymous with *Aecidium Pavettae* Berk., the host being *Pavetta indica* and not *Coffea arabica*.

Every contribution to the study of any *Hemileia* species must have a direct or a remote bearing on the control problem and be an improvement of our knowledge of the parasitism of *Hemileia vastatrix*, which causes the severe leaf rust disease of coffee. Numerous studies on the varietal resistance in coffee plants to *H. vastatrix*, spraying experiments in the control of the disease (Mayne, Narasimhan, et al., 1933), study of the physiologic races (Mayne, 1932), study of the importance of altitude to minimize the incidence of the disease (Dowson, 1921), and many other aspects of the problem clearly suggest the great interest evoked in the subject.

The species of *Hemileia* occurring on Rubiaceous plants have always been given special attention on account of their possible role in passing on the disease to coffee, which if true would be an important factor in the control of the coffee leaf rust. Such a possibility has, however, been negated by the work of Pole Evans (1907), Gyde (1932), and others who have carried out a series of cross-inoculation experiments. In the present study the authors are presenting a comparative account of the morphological characters of *Hemileia* species occurring on Rubiaceous plants in Mysore and the results of cross-inoculation experiments with the coffee leaf rust and other *Hemileia* species on Rubiaceae; this, in spite of being a repetition of the work of Pole Evans, Gyde, and others, would help to confirm the findings of those authors in South Africa by a study of the *Hemileia* rusts in Mysore, an important coffee-growing centre in India. Secondly, the identity of the *Hemileia* species on Rubiaceous plants has often caused confusion on account of the close similarity in the shape of the urediospores, the spore measurements being taken alone as the basis for differentiating the species. In the present study an attempt is made to take into consideration the structure of the sorus and the results of cross-inoculation experiments as additional evidences in separating the species of *Hemileia* on Rubiaceae in Mysore.

Material for the present study has been the result of collections for several years in the coffee plantations of Mysore. A careful watch had to be kept from time to time to study the occurrence of the telial stages, which are very scarce. *Hemileia Canthii*, unlike the other species of *Hemileia* on Rubiaceae, is mostly restricted to the dry regions. For microscopic studies bits of leaves with the sori were fixed in Allen's modification of Bouin's fluid and in formalin-acetic-alcohol. Sections of 8 to 10 μ thickness were cut and these were stained with Heidenhain's iron-alum haematoxylin with orange G as counterstain. The urediospores and teliospores readily germinated when placed on slides

in moist chambers and these were stained by the method suggested by one of the writers (Thirumalachar, 1940). For inoculation experiments the host plants were raised by cuttings in the case of *Randia uliginosa* and *Vangueria spinosa*, and from seeds in *Coffea arabica* and *Plectronia parviflora*. The plants to be inoculated were sprayed with water prior to dusting them with urediospores. In the majority of cases the inoculation experiments were carried out by the method described by Clinton and McCormick (1924) by maintaining entire leaves or fragments in Petri dishes. This method of inoculation has been found successful only with *Coffea arabica*, *Randia uliginosa*, and *Plectronia parviflora*, the leaves of which could be maintained in a fresh state for periods over 30 days, after which deterioration sets in.

(1) *Hemileia vastatrix* Berk. & Br.

The coffee leaf rust caused by *Hemileia vastatrix* first broke out in an epidemic form in Ceylon in 1868 and in the very next year it was recorded in Mysore. The possible spread of the disease from a focal point has been reviewed by Doidge (1926), Castellani (1937), and others. The rust is found in Mysore extensively on *Coffea arabica* and *C. liberica* (when it is cultivated) and to a minor extent on *C. robusta*. On many of the exotic species such as *C. eugenoides*, *C. excelsa*, *C. congensis*, &c., and wild species as *C. bengalensis* cultivated at the Government Coffee Experimental Station at Balehonnur, the rust occurs with equal severity.

The rust is foliicolous and strictly hypophyllous. The occasional occurrence of the rust on the berries and tender shoots reported by Butler (1918) has been observed by the writers on a *C. liberica* plant in Bangalore. The unripe berries show the rust pustules, the affected portions remaining discoloured even in the mature fruit. The rust severely infects also the cotyledonary leaves of quite a large number of seedlings, developed from dropped seeds, in the months of March–April. These may well be the means of the over-summering of the rust and most often they elude the attention of workers engaged in spraying against the leaf rust.

The morphology of the spore-forms and the details of development have been so well described by Ward (1882, 1882*a*), Delacroix (1900), Butler (1918), and others that it is superfluous to repeat them here. However, in the light of recent investigations on the morphology of the sori of rusts by numerous workers, some of the facts relating to the *Hemileia* might be stressed again. Only the developmental stages of *H. vastatrix* are given, for the other species of *Hemileia*, except for minor variations, show a similar type of development.

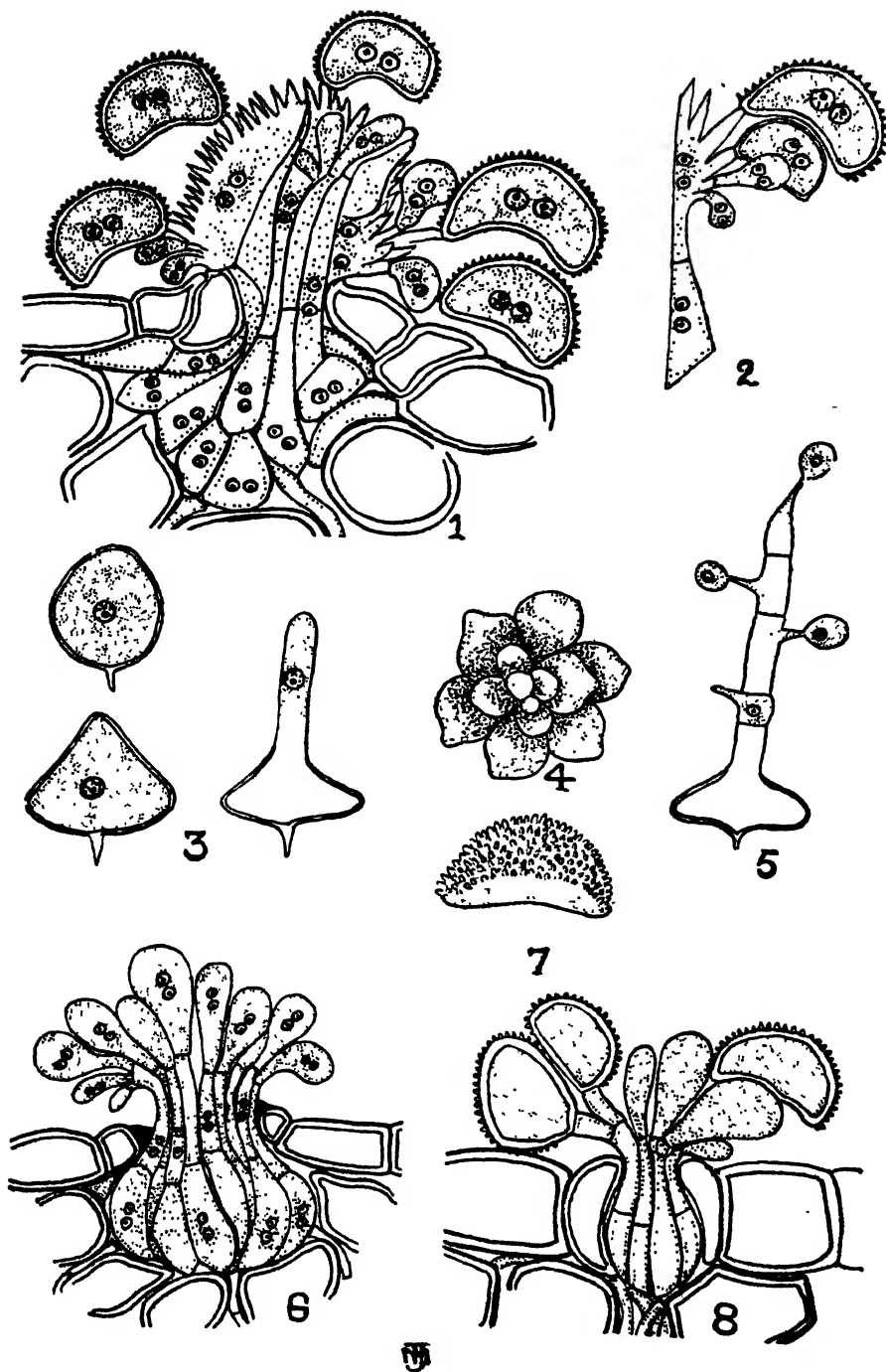
The rust is a hemi-form with uredia and telia borne on sori which are super-stomal. Prior to the formation of the sorus, strands of hyphae become grouped together in fascicles and these emerge from the stoma forming what have been termed 'stalks'. These hyphal strands appear to be separate at the beginning, but later show lateral coalescence and form a conical, head-like structure. In some of the species of *Hemileia*, these fasciculate hyphae expand and swell up. From each individual hypha which might more properly be called a sporophore,

a small stalk or sterigma is developed at the tip of which a young urediospore or teliospore is abstricted. Study of stained preparations reveals that the sporogenous structure has two nuclei and, in the process of spore formation, there is a conjugate division, the daughter nuclei migrating into the developing spore. By continued divisions of the nuclei of the sporophore more spores are produced in succession, resulting in a cluster of spores from each sporophore (Figs. 1 and 2). Such a method of spore formation has been described by Dietel (1923, 1928) and others. Dietel (1923) points out further that in the development of spores *Hemileia* could be compared with genera like *Cystospora* Butler, *Gerwasia* Racib., and others.

A point of comparison between the telia of *Hemileia* and that of *Gerwasia* might not be out of place. The genus *Gerwasia* founded by Raciborski (1909) has been described as having only telia, the spores of which are one-celled, thin-walled, pedicellate, and developed in clusters on a superstomal sorus composed of a single sporogenous hypha emerging through the stoma. The teliospores germinate immediately and are not resting spores. A comparison of the telium of *Gerwasia* and that of *Hemileia* indicates that the latter differs from the former only in there being several sporogenous hyphae emerging through the stoma and forming a fascicle, instead of the single one of *Gerwasia*. It might therefore be that the single sporophore with a cluster of pedicellate teliospores in *Hemileia* represents the entire telium of *Gerwasia*. In comparing the structure of the telium of *Blastospora* Diet., Mains (1936) refers to the sorus of *Hemileia* thus: 'in *Hemileia* the basal cells develop within the host and the pedicels of the teliospores project through the stoma'. The pedicels of the teliospores are, however, borne at the apex of the sporophores and the description of the telium of *Hemileia* by Mains does not give a true picture.

The uredia are hypophyllous on yellowish-brown leaf-spots. The urediospores are borne at the tips of the pedicels. Mature spores are bifacially ovate to reniform, the upper convex surface being covered with aculeate processes and the lower flat side being smooth. They measure $25-40 \times 20-30 \mu$. The teliospores, which had not hitherto been recorded in India, have been collected in great abundance. These had previously been recorded for Ceylon (Ragunathan, 1923, 1924), South Africa (Doidge, 1926), and other places. The teliospores first appear intermixed with the urediospores and later completely replace them, as has been observed in the collections made in the months of December and January. Ragunathan gives interesting data concerning the seasonal occurrence of the teliospores collected by him in Ceylon. He notes the occurrence of teliospores throughout the year except in the months of April, August, and October; he explains the absence of the teliospores in those three months as being due to the formation of new foliage in April and August and to heavy rains in October. In Mysore, however, the writers have been able to collect teliospore material mainly during the months from October to March.

The urediospores and teliospores germinate readily when placed in moist chambers. The germ-tubes of the urediospores become branched and develop



FIGS. 1-8. Figs. 1-5, *H. vastatrix*. Fig. 1, Uredium ($\times 800$). Fig. 2, Sporophore and spores ($\times 600$). Fig. 3, Teliospore ($\times 800$). Fig. 4, Teliospores ($\times 400$). Fig. 5, Germinating teliospore ($\times 800$). Fig. 6, Urediosorus of *H. Gardeniae-Thunbergiae* ($\times 450$). Fig. 7, Urediospore of *H. Thomasii* ($\times 800$). Fig. 8, Uredium of *H. Thomasii* ($\times 800$).
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appresoria at the tips. The teliospores are spherical to napiform (Fig. 3) with the hilum region appearing quite distinctly; the teliospores are never lunate or crescentic or triangular as in some of the other species of *Hemileia*. The germination of the teliospores has been described and illustrated by Ward (1882, 1882*a*), Delacroix (1900), and others. The entire process of germination and developing of basidiospores is completed within 6 hours. Prior to germination the apex of the napiform spore, or the portion of the teliospore opposite to the hilum, prolongates into a beak-like process and the promycelium is formed by further extension of this prolongation. There is no germ-pore through which the promycelium extrudes, and in this respect the germination of teliospores resembles those of *Maravalia*, *Catenulopsora*, and certain other rust genera. The fusion nucleus and the orange-yellow contents of the teliospore migrate into the promycelium which is cylindric, and for the most part does not become recurved (Figs. 3 and 5). After the complete depletion of cell contents the thin-walled teliospore becomes altered in shape. Following nuclear divisions the promycelium becomes four-septate and develops sporidia at the tips of sterigmata (Fig. 5). Each basidiospore receives a single haploid nucleus, but sometimes through another division it becomes binucleate.

Hemileia coffeicola Maubl. & Roger has been described as a species distinct from *H. vastatrix* by Maublanc and Roger (1934) from the French Cameroons, Africa. The rust was first described as *Uredo coffeicola* and it is stated to differ from *H. vastatrix* in the types of symptoms produced on the host and in the structure of the spores. Fortunately the rust does not occur in Mysore, and a careful search in the coffee-growing areas has failed to reveal it.

(2) *Hemileia Gardeniae-Thunbergiae* Maublanc & Roger

The rust on *Gardenia-Thunbergia* was first described by Hennings under the name *Uredo Gardeniae-Thunbergiae* P. Henn. It remained for the Sydows (1913) to point out that the rust was a species of *Hemileia*, but they identified it as *H. vastatrix*, thus making *U. Gardeniae-Thunbergiae* a synonym of *H. vastatrix*. Butler and Bisby (1930), Doidge (1926), and others followed the Sydows in the assigning of the rust on *Gardenia* to *H. vastatrix*. However, Maublanc and Roger (1934) by an examination of the specimens deposited in the Paris Museum concluded that the *Hemileia* species on *Gardenia* is distinct from *H. vastatrix*, and proposed the new name *Hemileia Gardeniae-Thunbergiae*. The study of the spore forms and cross-inoculation experiments carried out in the present study, on apparently the same rust occurring in Mysore on *Gardenia latifolia*, has lent support to the conclusions arrived at by Maublanc and Roger in considering *H. Gardeniae-Thunbergiae* as a species distinct from *H. vastatrix*.

The rust has been collected on the leaves of *Gardenia latifolia* in Balehonnur and other coffee-growing areas and near Burudalbore, Hassan, Mysore. The sori are strictly hypophyllous and the infection spot is not visible from the upper surface. As infection advances the entire surface of the leaf becomes

covered over with the powdery sori. In the developmental stages of the uredia and telia the rust resembles *H. vastatrix*. The sporogenous sporophores are slightly longer than those of *H. vastatrix* and are straight (Fig. 6). The urediospores measure $26-30 \times 18-24 \mu$ and are therefore smaller than those of *H. vastatrix*. Measurements of the urediospores collected at different places and seasons have consistently shown the same feature. The teliospores are associated with the urediospores, and collections made during humid weather showed numerous germinating teliospores. As in the case of *H. vastatrix*, the hyphal strands in the substomal space remain unaltered.

(3) *Hemileia* sp. on *Randia uliginosa*

A few leaves of *Randia uliginosa* collected by the writers near Nalur, Shimogga, Mysore State, had the yellowish rust pustules of *Hemileia* on the under surface of the leaves. Comparison with a species of *Hemileia* recorded by Thomas (1924) on the same host in Siddapur, Coorg, indicated that the two were identical. The sori are superstomal, minute, pulverulent, orange-yellow, and bear urediospores on the sporogenous sporophores (Fig. 8). The fasciculate sporophores are not as long as those of *H. Gardeniae-Thunbergiae* or those of *H. vastatrix* and *H. Woodii* Kalch. & Cooke. The hyphae in the substomal space remain unaltered as in the previous two species. The mature spores measure $21-6 \times 15-20 \mu$, and hence are slightly smaller than those of *H. vastatrix* and of *H. Gardeniae-Thunbergiae*.

It is interesting in this connexion to note that Thomas (1924), who first collected the rust, thought it to be *H. vastatrix* on account of the shape of the urediospores. However, Parendakar (1944), who again collected apparently the same rust, considered it to be *H. Canthii*, the collection having been made near Matheran, South India, on *Randia dumetorum*. He makes no mention of the characters of *H. Canthii* recently described by Thirumalachar (1943)—such as the possession of internal sori, &c.—as being present in the rust on *Randia dumetorum* collected by him, nor does he refer to the original finding by Thomas of apparently the same rust on *Randia uliginosa*. The identity of the species on the bases of the morphology of the sori and of inoculation experiments carried out by the writers will be considered later.

(4) *Hemileia Woodii* Kalch. & Cooke

H. Woodii has been known to occur on *Vangueria infausta* and *V. latifolia* in South Africa, but is present only on *V. spinosa* in Mysore. The rust is foliicolous with hypophyllous, superstomal sori which cover the entire surface of the leaves. The rust is collected best in the months of September to December, when the powdery yellow sori on the lower surface are conspicuous. The sporophores emerging from a stoma form a fasciculate column which is very much longer than in the rest of the species studied, measuring 35 to 60μ in length. Both uredia (Fig. 9) and telia have been observed, the urediospores measuring $26-38 \times 17-26 \mu$. The teliospores are mostly globose to spherical with many others lunate (Fig. 10). The fascicles of sporophores

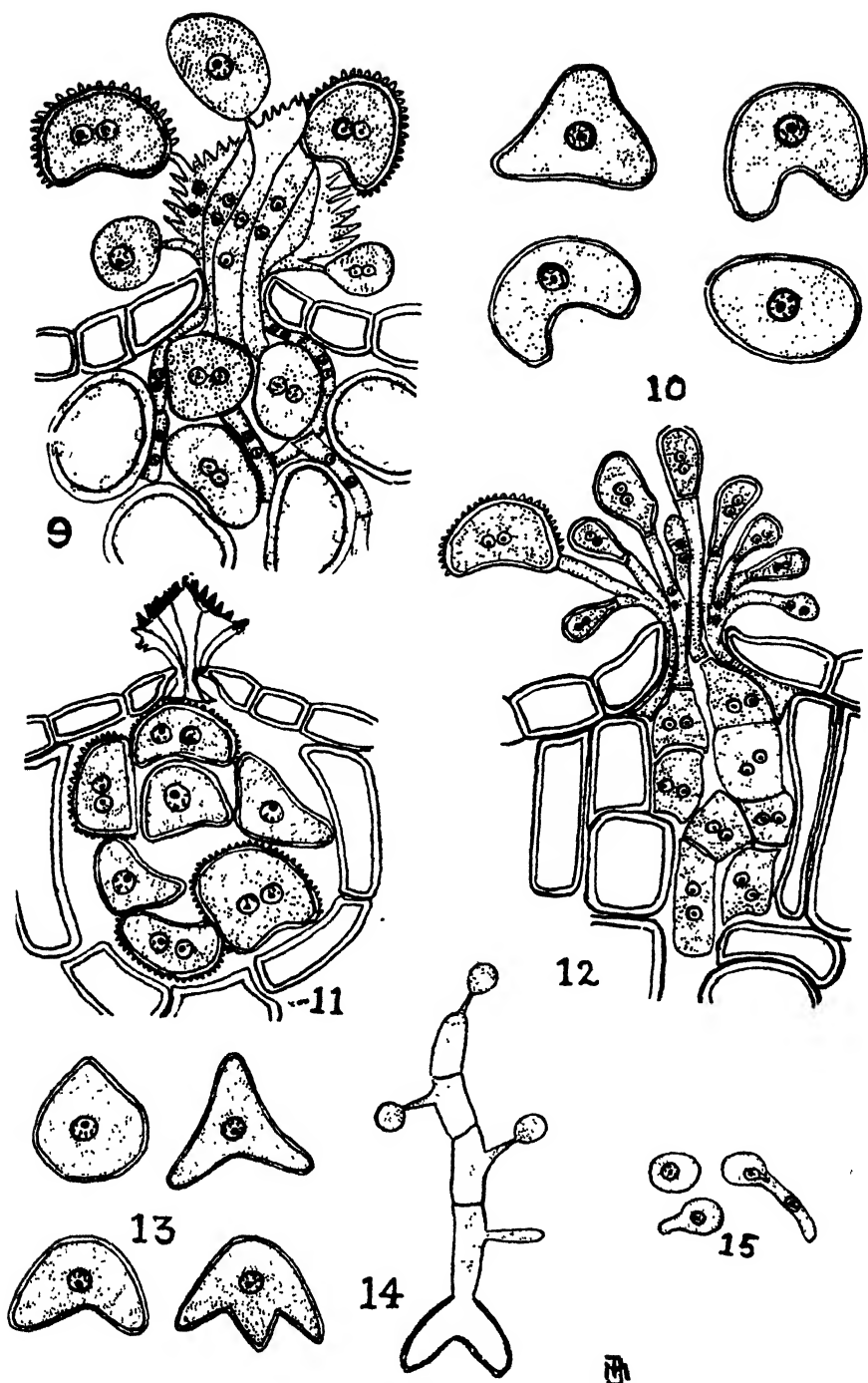
remain persistent even after the dispersal of the spores. Before the completion of the development of the superstomatal sori the mycelium in the substomal space enlarges, the nuclei becoming conspicuous and staining readily. The hyphae become closely septate and as development proceeds they round off into urediospores or teliospores forming from six to eight spores. The extent of development of these subepidermal spores is not as well pronounced as in *H. Canthii*.

(5) *Hemileia Canthii* Berk. & Br.

The species of *Hemileia* on *Plectronia* was first described by Berkeley and Broome from Ceylon and is known to have a wide distribution on *Plectronia parviflora* and *P. Rheedii* in South India. The rust mostly occurs in the dry scrub jungles rather than in the coffee-growing areas of Mysore. A morphological study of the rust was recently made by one of the writers (Thirumalachar, 1943). It has been pointed out that along with the development of the superstomal sorus the hyphae in the substomal space become conspicuous by their cytoplasmic contents which readily take stains (Fig. 12). They appear closely septate and later on the cells separate and round off into urediospores and teliospores, which resemble in all respects those produced normally on the superstomal sori. The spores produced beneath the epidermis are compactly grouped (Fig. 11) and become dispersed by the distension of the stomata. The urediospores are bilaterally ovate to reniform, with a large number of aculeate processes on the convex surface. The teliospores (Fig. 13) are spherical, triangular to crescentic as in *H. Woodii*. The teliospores germinate readily when placed in a moist chamber developing a promycelium bearing basidiospores (Fig. 14). The basidiospores germinate immediately (Fig. 15).

INOCULATION EXPERIMENTS

The object of these experiments was mainly to investigate the possible transference of *Hemileia* occurring on *Vangueria spinosa*, *Gardenia latifolia*, and *Plectronia parviflora* to coffee, and also to determine the role of the teliospores in the life-cycle of some of these rusts. The methods of inoculation have already been described. In the first series of experiments inoculations with urediospores were made; in every case the urediospore inoculations were made on the host from which they were collected. The results showed in all the cases that the urediospores could infect the host on which they were collected, and develop secondary uredia. For instance, in *Hemileia vastatrix* the first sign of infection appeared after the 9th or the 10th day as a small discoloured spot which gradually deepened into an orange-yellow patch. The sori emerged from the stoma and appeared as minute pin-heads bearing clusters of spores after the 15th day. The time taken for the development of the sorus is often extended to 25 days in the case of very old leaves. Similarly, in the case of *H. Woodii*, *H. Gardeniae-Thunbergiae*, and *H. Canthii* the urediospores developed secondary uredia.



FIGS. 9-15. Fig. 9, Uredium of *H. Woodii* ($\times 800$). Fig. 10, Teliospore of *H. Woodii* ($\times 800$). Fig. 11, Interval telium of *H. Canthii* ($\times 800$). Fig. 12, Early stage of external telium ($\times 800$). Figs. 13 & 14, Teliospore and germinating teliospore, *H. Canthii* ($\times 800$). Fig. 15, Germinating sporidia, *H. Canthii* ($\times 1000$).

Viable germinating teliospores of *H. vastatrix* were collected in abundance in the months of December and January. The teliospore clusters (Fig. 4) can be separated from the urediospores with which they are associated with the aid of a needle under the microscope. These were transferred on to young leaves of *Coffea arabica* which were sprayed with water. The teliospores readily germinated within 6 hours, developing basidiospores which germinated *in situ*. In spite of repeated trials with the basidiospore inoculations, no infection has been secured, confirming the assumption that the rust is not autoecious. Similarly inoculations with the basidiospores of *H. Canthii* on the leaves of *Plectronia parviflora* also gave negative results.

In the third series, cross-inoculation experiments with the urediospores of *H. Woodii*, *H. Gardeniae-Thunbergiae*, *H. Canthii*, and *H. vastatrix* were made. A susceptible variety of *Coffea arabica* was inoculated with the urediospores of *H. Woodii*, *H. Gardeniae-Thunbergiae*, and *H. Canthii*, the viability of the urediospores used for inoculation being tested in each case by placing them on slides in moist chambers. In all instances only fresh urediospores showing a high percentage of germination were used for inoculation experiments. In all cases the spores germinated, as could be seen under the low power of the microscope, but no entry of the germ-tubes through the stoma or any other sign of infection could be discerned. Similarly, inoculation experiments with the urediospores of *H. vastatrix* on *Plectronia parviflora*, *Vangueria spinosa*, and *Gardenia latifolia* gave negative results. Infection experiments by inoculating the urediospores of *H. Woodii* on *Plectronia parviflora*, *H. Gardeniae-Thunbergiae* on *Plectronia parviflora*, and *H. Woodii* on *Gardenia latifolia* have also failed. On the other hand, *H. vastatrix* on *Coffea arabica* readily infects *C. liberica*, *C. bengalensis*, *C. excelsa*, *C. eugenoides*, and other species of *Coffea*. Similarly *H. Canthii* on *Plectronia parviflora* can be passed on to *P. Rheedii*, and Gyde reports successful inoculation of *H. Woodii* on *Vangueria pygmaea* to *V. infausta*.

It becomes clear, therefore, that the various species of *Hemileia* on the members of the Rubiaceae are distinct from one another and under no circumstance are capable of passing from other genera on to *Coffea*. The present work is therefore another confirmation of the results of Pole Evans, Gyde, Thomas, and others who refuted Masee's opinion that the cultivation of coffee in the neighbourhood of *Vangueria* would be disastrous. Pole Evans was the first to point out that the rust on *Vangueria* would not pass on to *Coffea*; he was followed by Gyde (1932), Thomas (1924), and others who by a series of cross-inoculation experiments confirmed this fact. On the other hand, Gyde (1932) found that *Hemileia Woodii* on *Vangueria pygmaea* would infect *V. infausta*, while it would not pass on to any other host genus. Similarly it is well established that *H. vastatrix* would readily parasitize many species of the genus *Coffea* such as *C. arabica*, *C. liberica*, *C. robusta*, *C. bengalensis*, &c., and *H. Canthii* occurs both on *Plectronia Rheedii* and *P. parviflora*. From the above instances it is clear that each of the various species of *Hemileia* is restricted to a particular host genus.

In this connexion the identity of the rust on *Randia uliginosa* might be considered. On account of the similarity of the shapes of the urediospores, Thomas (1924) placed the rust under *H. vastatrix*, while Butler and Bisby (1931) placed it doubtfully under *H. Woodii*, and it was incorrectly assigned to *H. Canthii* by Parendakar (1944) when considering apparently the same rust on *Randia dumetorum*. The structure of the sorus in this rust resembles that of *H. vastatrix* and that of *H. Gardeniae-Thunbergiae*, since the hyphae in the substomal space remain unaltered. It has already been pointed out that in both *H. Canthii* and *H. Woodii* the substomal hyphae develop into urediospores and teliospores, a character not present in the rust on *Randia uliginosa*. Further confirmation has been obtained by inoculation experiments. While viable spores of *Hemileia* on *Randia* were not available, urediospores of *H. Canthii*, *H. Woodii*, and *H. vastatrix* were used for inoculation of young leaves of *Randia uliginosa*. These inoculations gave entirely negative results. These considerations, along with the fact that each species of *Hemileia* has so far been found to be confined to a particular host genus, indicate that the rust on *Randia uliginosa* cannot be accommodated under *H. vastatrix* or *H. Woodii* or *H. Canthii*, but represents an undescribed species. The name *H. Thomasii* is proposed, named after Dr. K. M. Thomas, Mycologist, Madras.

Hemileia Thomasii Thirumalachar & Narasimhan spec. nov.

Uredia hypophyllous on indeterminate pale leaf spots, sparsely distributed, yellow, very minute; urediospores borne at the tips of pedicels in clusters on fasciculate hyphae irregularly ellipsoid to reniform, convex surface densely and minutely aculeate, lower flat surface nearly smooth, measuring $21-6 \times 15-20 \mu$. Teliospores as yet unknown. Hab. On leaves of *Randia uliginosa* DC, Siddapur, Coorg, leg. K. M. Thomas,¹ 1924 (Type); Nalur, Shimogga, leg. Thirumalachar, 4/4/1945.

Urediiis hypophyllis, sparsis vel laxe aggregatis, pallide flavidis, minutissimis; urediosporis in apice hypharum fasciculatim erumpentium ortis, plus minusve reniformis $21-6 \times 15-20 \mu$, membrana pallide flavida, in superficie praeditis superiore aculeata, inferiore levi. Teliis adhuc ignotis.

GENERAL CONCLUSIONS AND SUMMARY

In the present study an attempt has been made to differentiate between the various species of *Hemileia* found on Rubiaceae in Mysore, on the basis of the morphology of the sori and cross-inoculation experiments. The fasciculate hyphae emerging from the stoma and bearing clusters of urediospores or teliospores reveal the relationship of *Hemileia* with other genera of the Uredineae such as *Cystopsora* and *Gerwasia*. The length of the sporophores, by themselves variable in a particular species, serves to add a differentiating character along with other features. For instance in the case of *H. Gardeniae-Thunbergiae* the sporophores are consistently longer than those of *H. vastatrix*.

¹ Grateful thanks are due to Dr. K. M. Thomas for placing at the disposal of the writers these valuable specimens.

The teliospores have been collected and germinated in all the species of *Hemileia* studied in the present investigation with the exception of *H. Randiae*. The teliospores are mostly subglobose to spherical, with the association of napiform spores in the case of *H. vastatrix*. On the other hand, many of the teliospores in *H. Woodii*, *H. Canthii*, and *H. Gardeniae-Thunbergiae* are crescentic, triangular tridentate, &c. Such types of spores have never been observed in the case of *H. vastatrix*. The teliospores in all cases germinate *in situ* producing sporidia. The view that sporidia produced in such abundance are functionless cannot be accepted without reservation. As in the case of many other genera of rusts where the pycnial and aecial stages were previously unknown, though discovered later, the genus *Hemileia* at present includes only hemi-forms on account of the lack of our knowledge of the life-cycle. None of those investigated are autoecious as indicated by sporidial inoculations of the respective hosts.

In the case of *Hemileia vastatrix*, which is widely distributed in the plantations at different altitudes, there is some evidence of the possibility of the over-summering of the rust in the uredial stage itself, without the teliospores being required for the completion of the life-cycle. On the other hand, in rusts like *Hemileia Canthii*, which occur only for a few months in the year and disappear soon after defoliation, the problem of annual recurrence would be of interest. The recurrence of the rust after a sufficiently long period of disappearance, and with a summer exposure long enough to kill any urediospore dropped in the vicinity, would, if observed, throw considerable light on the life-cycles of other species of *Hemileia*.

In conclusion the writers wish to express their gratitude to Dr. S. P. Wiltshire and Dr. G. R. Bisby of the Imperial Mycological Institute, Kew, for their kindness in going through the manuscript and giving the authors the benefit of their valuable suggestions.

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Water Movement in Submerged Aquatic Plants, with Special Reference to Cut Shoots of *Ranunculus Fluitans*¹

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With fourteen Figures in the Text

INTRODUCTION

THE problem of the 'transpiration stream' in submerged aquatic plants is one that has engaged the attention of a number of workers from time to time. In general, our knowledge of this phenomenon is of a qualitative rather than of a quantitative nature; whilst the experimental evidence indicates that such an upward flow of water may exist, there is little data on the magnitude of the current. Neither is there agreement as to the seat of the mechanism by which the upward flow is maintained; the term 'transpiration stream' is used by Arber (1920) to include water movement arising alike from root-pressure and from action in the leaves. In submerged plants the physical condition, i.e. evaporation from the leaves, which in aerial plants plays so large a part in the maintenance of the transpiration stream, is absent, and other mechanisms fundamentally of the secretory type must, if a similar upward flow exists, be in evidence. Herein lies the chief interest of the problem.²

That an upward current may exist in aquatics was shown by Unger (1862), who estimated gravimetrically the transfer of water from one vessel to another by whole plants of *Potamogeton crispus* and *Ranunculus fluitans*, threaded through a U-tube connecting the vessels. Over an experimental period of 8 days he observed the transfer of 1.6 and 0.8 gm. of water respectively by the two plants. A shoot of *P. crispus* from which the roots had been removed did not show this water transport.

Measurements of the water movement in cut shoots were attempted by Sauvageau (1891), who devised a simple form of potometer, adapted to under-water use, into which a cut shoot could be sealed. This apparatus showed an apparent slight uptake of water by the shoot, which in view of the apical openings at the leaf-tips was presumed to be given off again into the surrounding water. The apparatus has been criticized by Weinrowsky (1899) on the grounds that the cut end of the shoot was subjected to a slight hydrostatic pressure which would have tended to force water into it. It may be added

¹ Part of a Thesis approved for the degree of Ph.D. in the University of London, 1940.

² Especially as evidence is accumulating that the water relations of plant cells are not explicable entirely in terms of osmosis, but may involve metabolic activity of the protoplast Bennet-Clark et al. (1936, 1940).

that even if this pressure (a few centimetres of water) was insufficient to affect the true uptake of water by the conducting system of the shoot, it might still have tended to displace air from the intercellular space system, and this, if allowed to occur, would have been recorded as water absorption.

In view of the small volume changes it is required to measure, the direct physical measurement of water uptake thus presents certain difficulties. Another method of approach to the problem was utilized by Hochreutiner (1896), who observed the rate of ascent of eosin in the conducting strands of cut shoots of *Potamogeton* and other plants. The rates of ascent of the dye were low—a few centimetres per day. Similar experiments, but of much shorter duration, carried out by Thoday and Sykes (1909), on *Potamogeton lucens* growing in the river Cam gave much higher rates of uptake—5.7 to 9.5 cm. per minute—which were roughly proportional to the number of leaves on the shoots used. Removal of the leaves and the apical bud reduced the flow considerably, and it was concluded therefore that the uptake was due, principally at least, to the leaves. The data obtained by Thoday and Sykes are remarkable for the rapidity of the upward flow they indicate; this is some hundreds of times faster than the rates reported by Hochreutiner for the same species, though it should be noted that the experimental conditions in the two cases were very different.

It seems doubtful as to how closely the results of short duration experiments of this type can be related to the normal conditions prevailing in the vascular system; such experiments have been criticized by Barnes (1910) and Brown (1913), who attributed the results of them to purely physical phenomena not connected with the normal water movement (if any) at all. Some observations of the present writer also throw doubt on the value of experiments of this kind.

More recently (1932) Thut has attempted actual measurement of the amount of water passing through a submerged plant by means of a form of transpiration balance, fundamentally an elaboration of Unger's method. The apparatus consisted of two glass vessels containing water connected by a wide bent tube through which an aquatic plant could be threaded so that its roots lay in one vessel and its higher leaves and stem apex in the other. The whole apparatus was poised on the pans of a balance; the transfer of water from one vessel to the other was indicated by the apparatus becoming unbalanced, and the amount so transferred was deduced from the weight which it was necessary to add to one side of the balance to restore the original equilibrium. This form of apparatus has the advantage that it permits the use of complete plants as experimental material, but its value is limited by its lack of sensitivity. Owing to the necessarily heavy water chambers the balance used must be robust and hence comparatively insensitive. Thut found it necessary to use long experimental periods (1–4 weeks) during which time the plants were totally enclosed, so that although a flow of water from one vessel to the other apparently occurred, the amounts involved may have been far from normal.

In this connexion Thut's statement that attempts to use smaller vessels (so

as to increase the sensitivity of the apparatus) led to the death of the experimental material are relevant.

The rates of water transfer in complete plants (averaged over the total experimental period) were 4–11 mm.³ per hour, thus being of the same order as the earlier estimates of Unger. In addition, volumetric determinations were made (by the attachment of measuring tubes) of the exudation of water from the cut stumps of rooted plants, and of the uptake of water by the shoots cut from them. The rates of exudation of water from the rooted stumps were similar in value to the rates of water movement in complete plants, and the rates of uptake by cut shoots were $\frac{1}{4}$ th to $\frac{1}{20}$ th as great. Thut therefore concluded that in the plants with which he worked (*Elodea densa*, *Potamogeton* spp., *Ranunculus circinatus*, *Myriophyllum spicatum*, and others) the 'transpiration' phenomenon is in reality due principally to root pressure and not to special physiological activity in the leaves.

Thut's volumetric measurements of water movement may be liable to error for reasons which are explained below in connexion with experiments carried out by the writer. Apart from this, his comparisons of the water movement in complete plants (measured gravimetrically in the transpiration balance) and of the exudation of water by rooted stumps (measured volumetrically) appear to be invalid for another reason. In the only experiment with a complete plant in the transpiration balance which he cites in full, the water movement in the first 9 hours was 45 per cent. of that in the succeeding 22 days,¹ giving a mean hourly rate for the first 9 hours approximately 25 times that of the remainder of the experiment. In the experiments with rooted stumps the output of water, while remaining more or less constant for the first 8 hours, also fell off, virtually to zero, in 12–16 hours, and for comparative purposes the rate of the first 8 hours only was considered. The approximate agreement between the rates of water movement through complete plants, averaged over the whole experimental period, and the rates of exudation of water from rooted stumps of the same species is thus not of great significance.

The problem has also been attacked from the standpoint of water loss from the plant; thus Weinrowsky and von Minden (1899) observed the outflow of water from the leaf-tips of various normally submerged aquatics, e.g. *Potamogeton* spp., *Callitriche autumnalis*, the submerged form of *Littorella*, underwater leaves of *Alisma natans* and *A. ranunculoides*, by lifting them above the surface of the water in a saturated atmosphere. von Minden attributed this secretion to root pressure, as detached leaves did not show it, and treatment of the leaves with chloroform did not prevent the action. He also experimented with the leaves of many aerial plants which normally show guttation from leaf-tip hydathodes, and from the fact that killing the epithem with alcoholic mercuric chloride or copper sulphate did not affect the secretion von Minden concluded that the epithem is merely a passive filter and not a secretory tissue.

¹ See his Table I. There is obviously a misprint here; presumably the first entry should read Nov. 13, 8 a.m.

Culture experiments such as those of Pond (1905), Snell (1908), and Brown have also been held to throw some light on water movement in aquatics. These workers grew various aquatic plants under different conditions, e.g. rooted in soil and clean sand, and anchored above these substrata so that the roots did not penetrate them. In general the plants rooted in the soil showed better growth than the others, which was attributed to the effectiveness of the root system in the absorption of solutes and in providing a mechanism (i.e. an upward current in the xylem) whereby these are transported to the regions of growth. Brown regarded the soil as important as a source of additional carbon dioxide rather than of mineral salts, and adduced evidence in favour of this view.

Both Pond and Snell supported their conclusions by simple experiments demonstrating the uptake of salts by rooted aquatics, though in the light of more recent work clearly a distinction must be drawn between salt uptake and water movement (Hoagland, 1944, and work there cited). Pond also claimed to have shown that a root of *Ranunculus trichophyllus* absorbed 5 c.c. of water in each of two 24-hour periods, but as he apparently made only one experimental set-up, and was unsuccessful in his attempts to show simultaneous exudation of water from the leaves of the shoot, it is doubtful what value can be attached to this observation. Emphasis is laid below on the difficulty of making accurate measurements of water movement in aquatics, but nevertheless, if the 5 c.c. uptake was genuinely due to a water movement through the plant, its escape from the leaves should have been detectable.

The available evidence is thus rather fragmentary and inconsistent, and the problem seems to be one which would repay further study. A greater part of the work was, however, occupied with the development and investigation of the means of accurate measurement of the small volume changes observed. The points raised emphasize the difficulty of obtaining accurate data and the necessity for caution in accepting experimental evidence without rigorous investigation of the means by which it has been obtained.

THE EXPERIMENTAL MATERIAL

Ranunculus fluitans Lam., which was used almost exclusively for the work, has no floating or aerial leaves: the only part of the plant to appear above the surface of the water is the flower, so that for the greater part of the year it is totally submerged. The normal habit is that of a horizontal stem, rooted at the nodes in the lower part, but with the uppermost 5–6 internodes trailing freely in the stream. It was chosen for the work because it is robust and handles well, and was obtainable in quantity from some of the many streams of the river Colne in Middlesex. Whole plants were usually collected, the upper root-free portions being removed later for use. This minimized the growth of roots on the younger parts.

In transverse section the stem shows a ring of 5–8 vascular bundles, each enclosed in a well-marked endodermis. In each is a small number of xylem vessels and frequently a protoxylem lacuna. The phloem is well developed; phloem parenchyma is absent as in many other Ranunculaceae.

There is usually a large central air cavity in the pith, though this is very variable in size, and is interrupted at the nodes by diaphragms of unspecialized parenchyma, through which continuity of the intercellular space system is maintained. The pith cavity is often particularly large in the higher internodes of the shoots, where it appears to be of value in buoying up the flowers above the surface of the water.

At the node there is a complete ring of vascular tissue, from which the traces to the leaf and axillary shoot are given off. There are normally three bundles passing to the leaf, but the final ramifications of the latter, which are

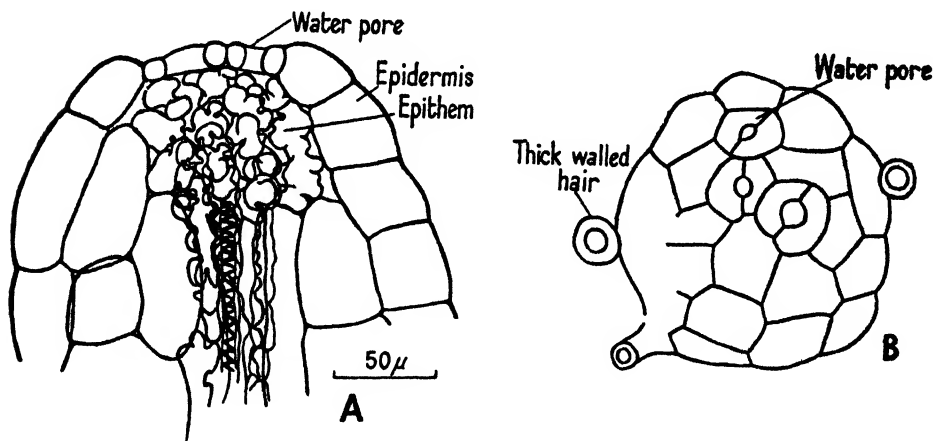


FIG. 1. A, Median longitudinal section. B, Surface section of hydathode of *R. fluitans*.

radially symmetrical in structure, are traversed by a single strand. By maceration of portions of the plant in concentrated sulphuric acid, as carried out by Schenk (1886), and by Priestley and North (1922), the endodermis can be traced as a continuous layer enclosing the vascular system of the leaf as well as of the stem, even into the final leaf segments.

The extreme tips of the latter are hemispherical in form. Each bears peripherally 2-4 thick-walled hairs and is occupied by an epithem hydathode.¹ The epidermis in this region contains a group of 3 or 4 water pores; underlying these is an epithem in which the vascular strand, here consisting of 1-3 spirally thickened vessels surrounded by a parenchyma sheath, ends. The epithem is similar in structure to that of many aerial plants: the cells are conspicuous by their marked lobing and absence of chlorophyll, and among them is an irregular system of intercellular spaces (Fig. 1). In old leaves the water pores break down and the intercellular space system becomes occluded by a brown gummy mass as described by von Minden for other species. In leaf-tips treated with concentrated sulphuric acid the Casparian band of the endodermis can be seen to become progressively fainter towards the extreme tip

¹ This term is used to distinguish this type of hydathode, where the bundle ends in a mass of small-celled delicate tissue with conspicuous intercellular spaces opening to the exterior, from the secretory epidermal hairs (hair hydathodes) which occur in many other plants.

and it is not distinguishable in the distal 1–2 mm. In all but very young leaves so treated the epithem and tip of the vascular strand also have the appearance of being suberized in that they resist the action of the acid, but this seems to represent an early stage in the deposition of the gummy matter which later completely blocks the epithem.

The structure of the hydathodes, which are presumably concerned in any water movement in the vascular system of the shoot, is discussed further on p. 112 in connexion with some experimental data to be described.

The ascent of dye (eosin) in cut shoots of Ranunculus fluitans

A considerable number of observations of this nature were made by a technique similar to that used by earlier workers.

The method, if used with due regard for its limitations, may give a rough estimate of the rate of water absorption, but it is subject to many disabilities. Its greatest disadvantage is that once the dye has reached any part of a shoot the experimental value of that part is finished. This, together with the difficulty of determining the ascent of the dye without cutting sections of the shoot, means that each shoot can be used once only, and comparisons of, for example, rates of ascent of dye under different conditions can only be made as between different shoots and not as between differences in behaviour of one shoot under different conditions. This introduced the complication of matching pairs of shoots for experimental purposes: while outwardly similar shoots may be selected without difficulty, they often show considerable internal differences, as for example in the size and number of vascular strands. Consequently when quantitative estimation depends on the length of stem or bundle stained by the dye it is liable to error on this account.

Plants were brought into the laboratory and, initially, cut shoots from them were used within a few hours. The experimental technique was to tamp the shoots into wide glass tubes with cotton wool and to seal the junction with a solution of gelatine melting at about 27° C. After the gelatine had set, the tubes were supported in a large sink full of water so that the shoots were completely immersed, any water in the tubes was then pipetted out and replaced by 2 per cent. eosin solution.

At the end of a suitable period, determined by trial and error, the shoots were removed and sectioned rapidly by hand from the apex downwards at intervals of 2–3 mm., and the points at which the dye became visible were noted in respect of each vascular bundle. The distribution of the dye in the bundles of an internode was often very variable. Thus not infrequently the stain would ascend the first internode in all bundles, traverse the second in one or two only, and then spreading round the nodal ring above resume its upward course in all the bundles. In the highest internode entered the dye might occur in one or two bundles only, vertically below the next leaf above.

In view of the irregularities of this sort the penetration of the dye was calculated as the total length of vascular bundle stained. Account was taken only of the stem bundles, the staining of the leaf traces being ignored. The

dye could, however, sometimes be traced into the finest ramifications of the leaf.

In this way the staining of the vascular bundles to a total length of 900–1,800 mm. in 30–45 minutes was frequently observed, corresponding to a rate of penetration of the bundle system as a unit of about 5–8 mm. per minute. It was estimated from the cross-sectional area of the xylem vessels and protoxylem canal at various points that this corresponds roughly to a volume uptake of about 6–10 mm.³ per hour.

As a parallel to experiments such as that carried out by Thoday and Sykes, the effect of the removal of the leaf-tip hydathodes on the ascent of the dye was investigated, by comparison of selected pairs of shoots, one member of each pair having had the tips (2–3 mm.) of all its leaf segments cut off 3–6 hours previous to the experiment. Considerable variability in the effect of this treatment was observed (see Table II for summarized data, and compare Table I where neither member of the pairs of shoots was mutilated). The variation within each of the first two columns of the tables is of no consequence, as the shoots varied in size and the experiments in duration. The only valid comparison is between pairs of corresponding figures in the two columns, which is expressed as a percentage difference in each case in the third column. From Table I the removal of the leaf-tips produced a mean decrease of 42 per cent. in the penetration of the dye: compare Table II where there is a mean difference of 6 per cent. between pairs of untreated shoots.

TABLE I

Total Length of Vascular Bundle stained by Eosin in Pairs of matched Shoots (untreated). The Distribution of the Members of each Pair in the two Columns was random

(a)	(b)	Percentage difference.
1,108	1,322	+19
741	424	-43
1,289	Nil	—
929	907	-2
1,219	835	-32
1,208	785	-35
1,522	880	-42
787	908	+15
1,208	Nil	—
571	951	+67
1,285	1,373	+7
866	301	-65
1,770	1,502	-15
1,074	845	-22
219	376	+75
		Mean - 6

This experiment thus suggests at first sight that there is a normal water uptake by cut shoots and that the removal of the hydathodes at the leaf-tips reduces it considerably. It was subsequently found, however, that shoots cut

and prepared in the laboratory not less than 10–15 hours before use usually showed very slight uptake of the dye, which considerably modifies the conclusions which can be drawn from the experiment outlined above. This point is discussed further below (p. 118) in connexion with experimental data obtained by other means.

TABLE II

Effect of Removal of Leaf-Tip Hydathodes on the Ascent of Eosin Solution in Cut Shoots

Total length of vascular bundle stained by the dye.		
Normal.	Tips removed.	Percentage difference.
1,376	536	—61
928	564	—39
904	640	—28
1,058	Trace	—
1,151	533	—54
1,084	623	—43
198	71	—64
Trace	Trace	—
Nil	57	—
Nil	1,068	—
133	Trace	—
1,201	Trace	—
1,175	810	—31
1,195	1,140	—6
1,805	737	—59
1,273	514	—60
1,156	Trace	—
1,660	Trace	—
926	Trace	—
1,876	492	—74
1,526	180	—88
1,636	1,401	—14
1,570	991	—37
1,451	1,100	—24
1,102	563	—49
980	534	—46
862	852	—1
1,729	1,265	—27
1,340	529	—60
1,086	852	—22
		Mean —42

The direct physical measurement of water uptake by cut shoots

In view of the many shortcomings of the eosin method, some apparatus for the direct measurement of the water uptake was considered essential; one which, moreover, would allow continuous observations of water movement to be made at intervals.

Volumetric rather than gravimetric methods seemed to be of more promise, so early experiments were directed towards an attempt to improve the potometer of Sauvageau. It was argued that the manifestly high sensitivity of that apparatus to temperature changes could be eliminated by the use of two water chambers of equal size, each fitted with a calibrated capillary tube for observa-

tion of volume changes of the contents, and so arranged that the lower cut end of the shoot lay in one vessel and the stem and leaves in the other. Movement of water through the plant would then appear as a decrease in the volume of the water in one vessel and an increase in that of the other, whilst temperature fluctuations, if common to both chambers, would produce identical changes in the volumes of their contents.

Different forms of apparatus were constructed on these lines and used in a large tank of water fitted with a stirrer, so that the temperature throughout was uniform at any given time, and any changes were slow and small. A considerable number of observations were made, using cut shoots, but the results were consistently inconsistent, the principal source of error being traced to the liberation of bubbles of gas from the plant. This is a point of some importance, which did not escape the notice of Sauvageau, but against which he nevertheless made no special provision. By keeping his experimental material at constant temperature and in weak diffuse light only, he claimed to prevent the escape of gas bubbles into his potometer. By using well-aerated water in the potometer he attempted to minimize the tendency to a gradual solution of the gas of the intercellular space system, as this resulted in injection of the air spaces, producing an illusory water absorption. The adequacy of these precautions is open to question. Thut's observations on the uptake of water by cut shoots must be accepted with reserve for the same reason. In the application of the simple potometer technique to aquatic plants it must be assured that no gas is given off by the plant into the potometer, and that no water passes into the intercellular space system.

The form of apparatus hitherto experimented with appeared to give no promise of compliance with these requirements and was abandoned in favour of a simpler form in which the compensation idea was abandoned and movement relative to the cut end only was measured, the leaves and apex being left free in a large volume of water. It was then possible to make the enclosed volume of the potometer very small so that temperature effects were negligible.

It was, however, still necessary to provide some means of dealing with the gas given off by the cut end of the shoot: this must not be suppressed, because unless gas is being evolved it is impossible to measure water uptake volumetrically, since otherwise there is no information available as to the position of the principal air/water surface in the intercellular space system. As long as gas bubbles are being given off, the air/water surface is always near the cut end of the shoot. If, however, gas production ceases, some of the air in the plant tends to become displaced by water and the pith cavity may become waterlogged, so giving rise to an illusory water absorption.

This effect has been observed and may lead to error. It provides the principal grounds for criticism of the apparatus of Sauvageau: the slight head of water to which his plants were subjected might have caused displacement of air in the plant by water.

As long as gas is being liberated from the shoot, errors from this source cannot arise, though it is clearly desirable that the evolution of gas bubbles from

the pith should be as smooth and regular as possible. Where the pith cavity is large, so that the liberated bubbles would normally be large and infrequent, the desired condition can be attained by lightly stuffing the pith cavity with wet unravelled string and then cutting off the excess with a sharp razor.

The evolution of gas by the plant into the potometer, which must be allowed to occur, introduces a complication to normal potometer technique. Attempts to meet this difficulty aimed at measuring, for each experimental period, (1) the overall volume change due to water absorption and gas production, and (2) the volume of the gas. The water absorption would then be obtained by difference. In practice, however, this proved unworkable. It was found that stopcocks had to be eliminated from the water-filled parts of the apparatus, as the grease from them tended to cause gas bubbles to stick to the walls of the tubes, and the separation and measurement of the gas volumes to a sufficient degree of accuracy could not be attained. Clearly, a systematic error in gas volume measurement might, in a form of apparatus based on this reasoning, give rise to an apparent water absorption proportional to the amount of gas given off, a rather attractive physiological correlation. This effect was, in fact, observed; each gas volume measurement was made as the length of a gas bubble in an otherwise water-filled capillary tube, and unless special precautions, which proved cumbersome and slow, were taken, the thickness of the water film lining the walls of the tube in the region of the gas bubble reduced the effective diameter of the tube, so that the volume of the gas (measured as the length of the bubble) was in error.

This system was therefore abandoned in favour of a simple design in which the gas produced by the plant was allowed to escape at an air/water surface within the potometer. At each reading of the volume of water in the potometer (made by observation of an index bubble in a capillary tube) this surface was set to a datum line, so that volume determinations should be affected as little as possible by variations in its position. The accuracy of the potometer was limited by the accuracy attainable in the resetting of the air/water surface, the limit being fixed by the smallest practicable diameter for the tube in which the air/water surface lay. If a very narrow tube was used, bubbles of gas from the plant, instead of breaking surface and escaping, tended to form airlocks in the tube, so invalidating the measurements.

The potometer is shown in Fig. 2. The essential part of it (for the measurement of water uptake) is the region ABCDEF. The plant shoot was attached at C by means of a piece of cycle-valve tubing, and measurement of water movement was made by means of an index bubble in the region DE. This consisted of a carefully calibrated capillary tube of approximately 2 mm.² cross-sectional area, and carried a scale. The free water surface, at which bubbles of gas given off by the plant escaped from the apparatus, lay in the region AB. Before each reading of the position of the index bubble in DE the meniscus in AB was set to a datum scratch near B; under this standard condition, therefore, water uptake by the plant registered as a movement of the index bubble in DE, and was not directly effected by gas production by the plant.

A practical lower limit to the diameter of AB was set by the size of the gas bubbles given off by the plant. It was generally possible to ensure, by the method outlined above (p. 100), that the gas bubbles given off were small enough to rise in a tube of 2 mm.² c/s area without forming air-locks in it: both AB and DE were therefore made of this size tube.

In use the apparatus was filled with water and clamped with the region BF submerged. An air bubble was then introduced at F (to serve as an index

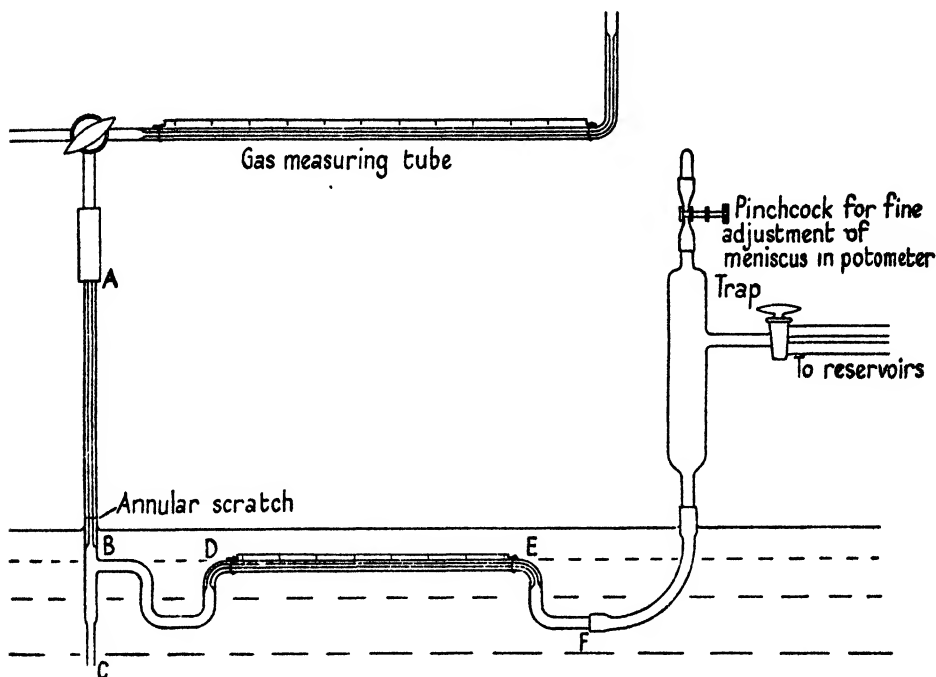


FIG. 2. Potometer for use with aquatic plants.

bubble) and F was connected to a water-filled trap, connected in turn through a two-way stopcock to two reservoirs, one above and one below the level of the water in the experimental tank. Thus by manipulation of the stopcock a current of water in either direction could be produced in the potometer. The plant shoot was then attached at C and any air in the region of C displaced upwards to escape at A; the vertical region below D, being of wide bore, prevented the index bubble in DE from being carried over also. The meniscus in AB was then lowered to B and after an interval for the drainage of the water films lining the region AB the apparatus was ready for use. Observations were carried out in a glass tank, well stirred, and illuminated by a 200-watt water-cooled lamp which could be raised or lowered to alter the light intensity. The three-way stopcock and gas-measuring tube shown attached to the potometer at A were added for approximate measurements of the volume of gas given off by the plant, the gas being allowed to displace a short water column in

the calibrated tube. When this was not required, the stopcock allowed the gas to escape directly to the atmosphere.

Water movement with respect to the cut end of the shoot

Using this potometer it was found that measurable water movement could be detected with many, though not all, cut shoots, but the direction of the movement was not constant. Whilst some shoots showed absorption, others exuded water into the potometer.

Rates of water absorption (by shoots 25–60 cm. long and bearing 6–9 leaves) were initially of the order of 1–10 mm.³ per hour, though the rate fell off with time, tending towards zero in 5–8 hours. Rates of negative absorption were somewhat less, though of the same order, being 0.7–5 mm.³ per hour initially, decreasing to zero in 1–4 hours. Not infrequently the initial negative absorption of water by a shoot freshly set up in the apparatus would die away in an hour or two, to be reversed in direction. The reverse order, i.e. absorption followed by exudation, was never observed.

TABLE III

'Negative Absorption' of Water from Shoot Segments (approx. 10 cm. in length) due to Air Stream in intercellular Space System (see also Fig. 3)

	Amount exuded (mm. ³).	Hours.	Initial rate (mm. ³ /hr.).
Shoot segment normal	2.4	5	0.6
	3.0	4	1.0
	2.8	3	1.5
	4.4	5	1.5
	5.4	3.5	2.0
	6.8	4	2.4
Shoot segment injected, but free water removed from pith cavity	25.0	4.0	18.8
	26.7	4.0	18.0
	27.5	3.5	21.9

While there seemed to be no *a priori* reason why this negative absorption should not occur, it was unexpected, and the operation of the potometer was tested with an 'artificial bubbler' device which had been found of great value in investigating the behaviour of other forms of potometer. In brief, this consisted of a short length (10 cm. or thereabouts) of stem of *R. fluitans*, one end of which was connected to a finely adjustable source of air (from an aquarium aerator). The other end of the stem segment was then treated as if it were the end of a leafy shoot and sealed into the potometer in the normal way. It was thus possible to produce at will, in the potometer, a gas stream simulating that of a photosynthesizing shoot with a minimum of complication by water absorption by the shoot.

As a result of several tests of this kind, it became evident that the passage of air through a piece of stem in this way was commonly accompanied by a displacement of water from the stem into the potometer. The amount of water so displaced from a length of stem of 8–10 cm. was of the order of

2–4 mm.³ in 2–5 hours. At the end of this time the rate of exudation had usually decreased almost to zero.

This effect was obtained with stem segments which had been cut and prepared 24 hours before use. It could be minimized by blowing air vigorously through the segment for an hour or two, and could be greatly enhanced by injection of the intercellular space system of the shoot segment. Injection was attained by keeping segments in boiled, cooled distilled water under reduced pressure for 40 minutes. At the end of this time they were markedly

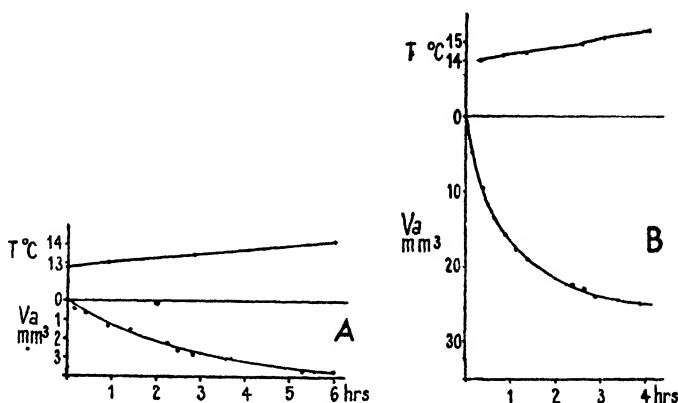


FIG. 3. 'Negative absorption' of water from a short length of stem under the influence of an artificial air flow through the intercellular space system. A, Stem segment as cut from the plant. B, Stem segment after injection of the intercellular spaces with water.

heavier and more translucent than usual, and clearly contained a comparatively large amount of water in their intercellular space system.¹ A number of such injected shoot segments, when connected to the potometer in the 'artificial bubbler', all showed rates of exudation of water initially 6–8 times normal, and exuded 24–7 mm.³ of water in 3½–5 hours. At the end of this time exudation was still going on though only approximately at the rate characteristic of a freshly set-up, normal shoot segment (see Fig. 3 and Table III).²

The water exuded in this way clearly came largely from the intercellular space system; the amounts involved were too great to be held in the vessels and xylem canals, and the air pressures used were insufficient to displace water from the xylem system—at least, bubbling from the vascular system was not observed. The displacement of water from the intercellular space system was presumably due to frictional drag between the air current and the water films of the intercellular spaces, and ceased when equilibrium between this drag and the surface-tensional forces of the water films had been reached.

¹ i.e. in the intercellular spaces of the outer pith and cortex. The main pith cavity, though the first to become injected, was similarly the first to lose the water it contained, and this was removed before the shoot segment was attached to the potometer.

² V_a in the figures refers to volume of water absorbed or exuded (absorption being regarded as positive), and V_g to the volume of gas produced by the shoot where this was measured.

Though this equilibrium was attained in a short length of stem in a few hours, in a complete leafy shoot it would not be possible to determine if or when it had been reached, so that measurement of water uptake by cut shoots must be liable to errors on this account. The occurrence of exudation and absorption by cut shoots, and the change-over from the former to the latter which has been observed, can be explained in terms of relative rates, or changes in rates, of absorption, presumably by the vascular system, and exudation, from the intercellular spaces of the non-vascular tissues of the shoot. That these two processes should go on simultaneously, as appears to be the case, is an interesting sidelight on the endodermis as a physiological barrier.

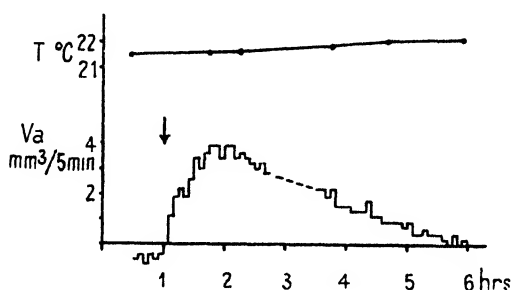


FIG. 4. Typical water uptake by a cut shoot stimulated by 0.2 per cent. sodium bicarbonate solution. This shoot showed negative absorption in tap-water. The arrow indicates the time of transfer to bicarbonate.

The Effect of Solutes on the Uptake of Water

It was sometimes found that, under the experimental conditions prevailing, shoots would not give off gas bubbles at the cut end. The liberation of free gas from the cut end of a shoot of an aquatic is dependent on a variety of factors; besides the intensity of illumination the amount of dissolved air in the water and the rate of water movement relative to the plant are involved. In these experiments it was found that bubbling could usually be restored by the use of a stronger light, but as this was objectionable on account of its heating effects, other means to the same end were sought.

Following the practice of workers on assimilation in aquatics the bubbling rate was increased by the addition of sodium bicarbonate to the water in the tank. This had the advantage over the use of carbon dioxide as such, of being more easily reproducible and more constant in its action over long periods. A 0.2 per cent. solution was found to be adequate for the purpose in view, a concentration well within the range (up to 0.4 per cent.) stated by James (1928) to have no deleterious effect on the plants with which he experimented.

In addition to the expected effect on photosynthesis, sodium bicarbonate was found to have a remarkable stimulatory effect on the uptake of water by cut shoots, this being increased to 10–15 times the rate commonly observed in tap-water.

It was found that shoots placed in 0.2 per cent. bicarbonate solution, which also filled the potometer (or transferred to this solution from tap-water after

having been attached to the potometer), showed a rapid acceleration of water absorption up to rates of the order of 25–50 mm.³/hour. This high rate remained fairly constant for 1½–2 hours and then slowly declined almost to zero during the next 1–3 hours (see Fig. 4). This decline in the rate of absorption, following the steady period, took place irrespective of whether the shoot was in tap-water or bicarbonate solution. Thus (see Fig. 5) a shoot *A* was placed in bicarbonate solution and its absorption rate followed until it had

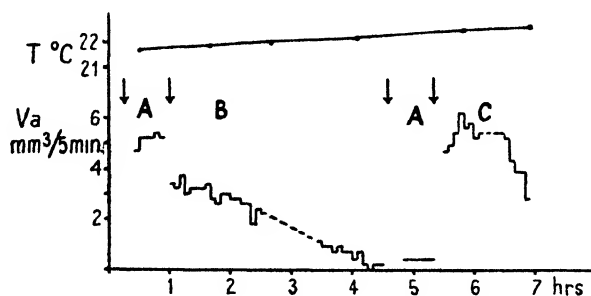


FIG. 5. The decline in response to bicarbonate solution. For explanation see text.

reached the peak; it was then transferred to tap-water. A second shoot *B* was then stimulated in the bicarbonate solution and when its absorption had declined almost to zero *A* was replaced in the solution; it, too, showed a low rate of uptake with no tendency to rise. Yet a third shoot *C*, fresh from tap-water, reacted to the same solution just as *A* had done initially. Replicates of this experiment are summarized in Table IV.

TABLE IV

Decline in Response of Shoots to the Stimulus of 0.2 per cent. Sodium Bicarbonate Solution: Rates of Water Uptake in mm.³/5 min. (see also Fig. 5)

	<i>A</i>	<i>B</i>	<i>A</i> replaced.	<i>C</i> fresh from tap-water.
1.	4.7, 5.2, 5.2, 5.4, 5.2 . . .	3.4, 3.2, 3.7, 3.0, 3.2, 3.2, declining to 0.6, 0.2, 0.0, 0.2, 0.2, 0.2 . . .	0.4, 0.4, 0.4, 0.4 . . .	4.7, 4.9, 5.4, 6.2, 5.6 . . .
2.	4.5, 4.5, 4.5, 4.9, 4.7	4.1, 4.1, 4.3, 4.5, 4.3, 4.5, 4.5 . . . declining to 0.6, 0.9, 0.6	1.3, 1.3, 1.5, 1.3, 1.7 . . .	2.8, 2.4, 3.0, 3.2, 2.6, 3.4 . . .
3.	4.3, 3.9, 4.7, 4.3, 4.5 . . .	4.3, 4.9, 5.6, 5.4, 5.8, 5.6, declining to 0.4, 0.4, 0.6, 0.2, 0.4, 0.2	0.4, 2.2, 0.9, 2.2, 0.9, 2.2, 2.2 . . .	1.9, 2.4, 2.6, 2.2, 2.6, 2.8

A similar result was obtained in a different way by observing the absorption rates of shoots transferred from tap-water to bicarbonate solution and back again repeatedly at intervals of about 30 minutes. In this way the normal

stimulation curve could be traced out in skeleton form (see Fig. 6 A and Table V).

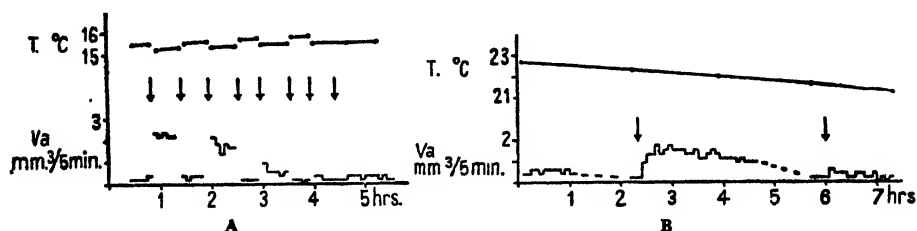


FIG. 6. A, Decline in response to 0.2 per cent. bicarbonate solution as shown by intermittent stimulation: shoot transferred from tap-water to solution and back again repeatedly (first seven arrows). The 8th arrow indicates a doubling of the bicarbonate concentration; note slight response. (See also Table V.) B, Shoot showing well-marked absorption in tap-water, and normal response to bicarbonate solution (1st arrow). The response to a doubling of the concentration (2nd arrow) was slight. Compare Fig. 10 where the concentration was doubled earlier, during the first response.

TABLE V

Intermittent Stimulation of Water Uptake ($\text{mm}^3/5 \text{ min.}$) by 0.2 per cent. Sodium Bicarbonate (see also Fig. 6 A). W = water, B = NaHCO_3 solution

	W	B	W	B	W	B	W	B
(1)	—0.2	2.8	0.9	2.2	0.2	1.5	0.2	1.1
	Nil	2.8	0.6	2.6	0.2	1.7	0.2	1.3
	—0.2	3.2	0.6	3.0	0.4	1.9	0.2	1.5
	Nil	3.9	0.9	2.6	0.2	1.7		1.3
	—0.2	4.3	0.6	2.4	0.2	1.7		1.3
		4.5	0.6	2.6	0.2	1.5		
						1.5		
						1.9		
			0.4					
			0.4					
			0.4					
			0.4					
			0.2					
(2)	0.2	2.4	0.4	2.2	0.2	0.9	0.2	0.4
	0.2	2.2	0.2	1.9	0.2	0.6	0.2	0.4
	0.2	2.4	0.4	1.5	0.2	0.6	0.2	0.2
	0.4	2.2	0.4	1.7	0.2	0.4	Nil	0.2
		2.2	0.4	1.7		0.4	0.2	0.2
						0.6		
(3)	0.8	4.2	0.8	3.6	0.4	2.0	0.6	0.4
	1.0	4.2	1.0	3.2	0.4	1.2	0.4	0.6
	0.8	4.4	0.8	3.6	0.2	1.4	Nil	0.4
	0.8	4.6	0.6	3.6	0.2	1.4	0.2	0.4
	0.8	4.8	0.8	3.0	Nil	1.4	Nil	0.4
	0.8	5.2	1.0					
	0.8	5.0						
(4)	1.1	6.0	1.7	3.7	Nil	1.7	0.2	0.6
	0.9	6.4	1.3	4.1	0.2	1.9	Nil	0.9
	1.1	7.7	1.1	4.1	0.4	1.5	0.4	0.4
	1.1	7.3	1.3	3.7	Nil	1.7	Nil	0.6
	1.1	7.5	1.3	3.4	0.2	1.7	0.2	0.6
	1.1	7.3	0.9					

The power of response to the bicarbonate solution is thus gradually lost irrespective of whether the shoots remained in it or in tap-water, and furthermore, when the response had died down, a doubling of the concentration of the bicarbonate (to 0.4 per cent.) had a very small effect (see Fig. 6 and also Fig. 8). Plant shoots did not regain the power of response to 0.2 per cent.

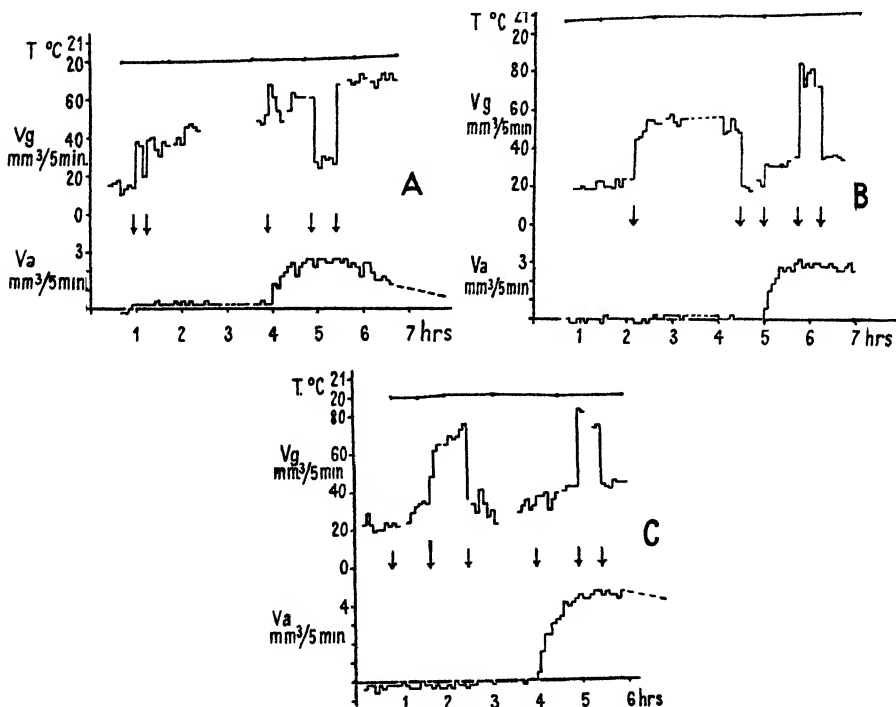


FIG. 7. A, Shoot showing absorption in tap-water, and normal response to 0.2 per cent. bicarbonate. The first two arrows indicate addition of CO_2 to maintain the bubble rate. The 3rd arrow indicates addition of bicarbonate, and the 4th and 5th arrows changes in the light intensity (reflected in the bubble rate) which had no influence on water absorption. B, Shoot showing negligible water absorption, and response to 0.2 per cent. bicarbonate (1st and 2nd, and 4th and 5th arrows indicate changes in light intensity; the 3rd arrow marks addition of bicarbonate). C, Shoot showing negative absorption in tap-water, declining to zero, with normal response to bicarbonate (1st arrow, addition of CO_2 ; 2nd and 3rd, 5th and 6th arrows, changes in light intensity; 4th arrow, addition of bicarbonate).

bicarbonate after 20 hours in tap-water following the first stimulation, though some recovery was observed after 2-4 days.

Whilst increases in the concentration of the solution were thus almost ineffective if applied after the first response had decayed, if applied earlier, whilst this was still at its height, they produced a further stimulation. Shoots were placed in 0.2 per cent. bicarbonate, and as soon as the absorption rate had reached a steady value were transferred to a 0.4 per cent. solution. A further increase in the rate of absorption, of about 1.6 times, occurred, was

maintained for a period, and was then followed by the normal gradual decay (Fig. 10 A).

At higher concentrations the response to the second stimulus became progressively more intense and of shorter duration. Thus shoots stimulated initially in 0.4 per cent. bicarbonate and transferred to 0.8 per cent. bicarbonate showed only a transitory response to the higher concentration and a return within 10–15 minutes to the rate characteristic of 0.4 per cent. bicarbonate. In the highest concentration applied (2 per cent.) the peak of the absorption

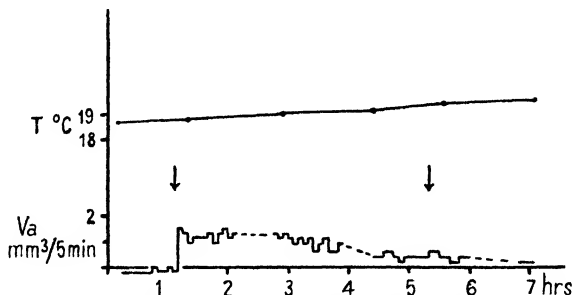


FIG. 8. Response of shoot, showing initially negative absorption, to 0.14 per cent. sodium chloride (1st arrow). The 2nd arrow indicates a doubling of the salt concentration.

curve was very short-lived, readings at one-minute intervals showing a decline after 2–3 minutes. See Fig. 10 B.

The mechanism of this stimulus is not clear. The concurrent stimulation of photosynthesis (as indicated by an increase in the rate of gas production) was not directly concerned, as similar changes in the bubbling rate could be produced by the addition of carbon dioxide to the water (from a 'Sparklet' siphon), or by changes in the light intensity, without a corresponding effect on the rate of absorption (see Fig. 7 A).

Furthermore, similar stimulatory effects have been obtained with other solutes—sodium chloride (Fig. 8), calcium chloride, and glucose. The effect would therefore appear to be largely an osmotic one, the external solution withdrawing water from the tissues, which loss is made good by absorption from the potometer, though there are aspects of the phenomenon not wholly satisfied by this view. It is noteworthy that the stimulatory powers of equimolar solutions of sodium bicarbonate and sodium chloride were found to be markedly different, though their plasmolysing powers with respect to leaf tissue were found to be very similar, the chloride being slightly the more effective in this respect.¹ The difference in the stimulus brought about by these two salts was first noticed as a generally poorer response of the plant to the chloride compared with the bicarbonate; subsequently shoots were transferred from one solution to the other and the response to the chloride was found to be about 40 per cent. of that to the bicarbonate (see Fig. 9 and Table VI).

¹ Thick longitudinal sections were tested for plasmolysis in solutions of both salts. The osmotic pressure of the tissue was approximately that of 0.2 M sodium chloride.

As to whether the water absorbed from the potometer was in fact passed through the plant to the exterior, or was held within the plant shoot (always a point of some uncertainty in potometer experiments), there is little evidence. It was noted, however, that shoots stimulated in 2 per cent. (= 0.24 M) bicarbonate became flaccid, and after 10 minutes (i.e. towards the end of the transient response to the high concentration; see Fig. 10 B) the leaf tissue was found to be generally plasmolysed, and there was no sign of recovery

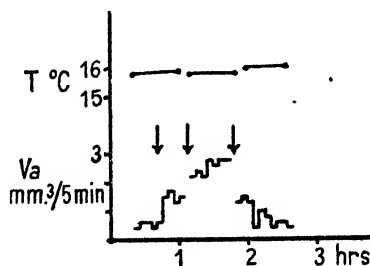


FIG. 9. Response of shoot to stimulation in 0.14 per cent. sodium chloride (1st arrow), transfer to 0.2 per cent. sodium bicarbonate (2nd arrow), and back to chloride (3rd arrow). See also Table VI, (These salt concentrations are equimolar.)

during the next 2 hours. Under these conditions, therefore, water loss by the shoot to the exterior was definitely occurring and the absorption from the potometer was presumably stimulated by this loss. These plasmolysed shoots were subsequently kept in running tap-water for 4 days, long after they had become deplasmolysed and had apparently regained their normal turgor. Even so they showed no response in water uptake to immersion in 0.2 per cent. bicarbonate, further evidence of the prolonged effect of the stimulus.

Though the effect thus appears not to be wholly an osmotic one, and other possibilities must be considered, e.g. the known salt effects on respiration

TABLE VI

Comparison of the stimulatory Effect on Water Uptake (mm.³/5 min.) of equimolar Solutions of Sodium bicarbonate and Sodium chloride. Four Periods in Sodium chloride (at the maximum Rate attained in this Solution), subsequently in Sodium bicarbonate and then after return to Sodium chloride

Shoot.	NaCl.	NaHCO ₃ .	NaCl.
1	0.4, 0.4, 0.6, 0.6	1.1, 1.1, 1.5, 1.7, 1.5, 1.7, 1.5	—
2	0.4, 0.4, 0.4, 0.4	1.3, 1.5, 1.5, 1.5, 1.7, 1.5, 1.5, 1.5	—
3	0.6, 0.6, 0.9, 0.9	0.9, 1.5, 1.3, 1.7, 1.7, 1.7, 1.9	1.3, 1.1, 0.6, 0.9, 0.6, 0.6
4	0.4, 0.4, 0.6, 0.6	1.1, 1.3, 1.3, 1.9, 1.7, 2.2, 1.9, 1.9	1.1, 0.6, 0.6, 0.4, 0.4, 0.6
5	1.5, 1.7, 1.3, 1.5	2.2, 2.4, 2.2, 2.8, 2.6, 2.8, 2.8	1.3, 1.5, 0.6, 0.9, 0.6, 0.4, 0.6

(Lyon, 1921; Steward and Preston, 1940) and permeability (Baptiste, 1935), the difficulty of finding some factor, other than osmotic pressure, common to the solutions used in the experiments is an obstacle to this view.

In considering the nature of the response to the solutes it is important to note that there are really two separate effects. There is a short-lived phase, evident as a rapid increase in water uptake when the plant is immersed in the solution and an equally rapid fall when it is removed from it to tap-water (see

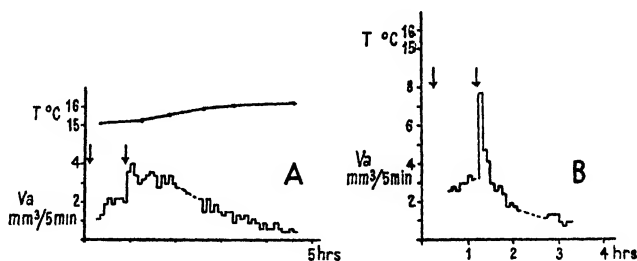


FIG. 10. A, Response of shoot to 0.2 per cent. bicarbonate (1st arrow) and doubling of concentration (2nd arrow) whilst the first response was still at its peak. Compare Fig. 6. B, Response of shoot to 0.2 per cent. bicarbonate (1st arrow) and a transfer to 2.0 per cent. bicarbonate (2nd arrow).

Fig. 6). In addition there is a long-term phase: a gradual loss in the power of the plant to respond to the stimulus, whether it be kept in the solution or in tap-water. The short-lived phase may well be osmotic, because the absorption rate follows so closely on immersion in or removal from the activating solution. The long-term phase must, however, be different. It is initiated within 30–40 minutes of immersion of the plant in the solution, but does not become apparent until approximately 1–2 hours later. After that it may persist for a considerable period. Clearly some unappreciated factor in the water relations of the plant is here involved.

Though the mechanism of the action of sodium bicarbonate and other solutes in stimulating the rate of water uptake by cut shoots had thus not been elucidated, it nevertheless appeared that the high values of water uptake which could be induced in this way might be of interest in other directions, the bicarbonate treatment being used as a means of obtaining a rate of water absorption high enough to be experimented on in other ways. Its value in this direction was limited by the comparatively short duration of the effect, but this period was long enough for the application of some experimental treatments of interest.

The effect of the removal of the leaf-tips on the water uptake of shoots in sodium bicarbonate solution

It was shown earlier that the removal of the tips of the leaf segments produced a marked reduction (about 40 per cent.) in the rate of ascent of eosin solution in the vascular system of freshly cut shoots. By the use of shoots

previously stimulated in 0.2 per cent. sodium bicarbonate solution this effect of leaf-tip removal on water uptake was confirmed by direct measurement.

Each shoot was set up in the potometer in tap-water (or tap-water plus carbon dioxide to ensure adequate bubbling). Sodium bicarbonate was then added to the external water, and when the rate of uptake by the shoot had reached its steady value the tips of the leaf segments were cut off under water

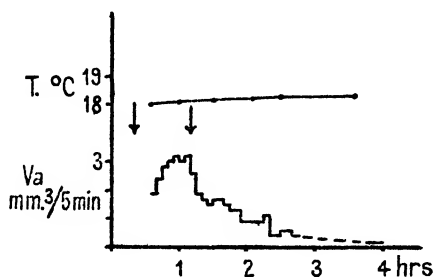


FIG. 11. Uptake by shoot in 0.2 per cent. bicarbonate (1st arrow) and effect of removal of leaf-tips (2nd arrow). (See also Table VII.)

with scissors without otherwise disturbing the shoot. The rate of water absorption fell almost immediately to approximately half its previous value, thus paralleling closely the results of the experiments with eosin. The numerical data are summarized in Table VII and a representative series of observations on one shoot is shown in graphical form in Fig. 11. This shows that

TABLE VII

The Effect of Leaf-Tip Removal on the Water Uptake ($\text{mm.}^3/5 \text{ min.}$) of Shoots in 0.2 per cent. Sodium bicarbonate for three Periods preceding and three Periods following Removal of Leaf-Tips. One intermediate transitional Period has been omitted

Shoot.	A Before tip removal.	B After tip removal.	B/A Ratio for total 15 min. period.
1	3.2, 3.0, 3.4	1.9, 1.7, 1.5	0.53
2	3.2, 3.4, 3.2	1.3, 1.3, 1.1	0.38
3	1.3, 1.5, 1.3	0.6, 0.6, 0.6	0.44
4	1.3, 1.1, 1.3	0.6, 0.6, 0.6	0.49
5	5.2, 5.2, 4.9	2.8, 2.2, 2.6	0.50
6	1.7, 1.9, 1.7	1.1, 1.1, 0.9	0.58

Mean 0.49

the fall in absorption rate following removal of the leaf-tips was much more abrupt than, and quite distinct from, the gradual decline which normally occurred on prolonged immersion in the bicarbonate solution. Moreover, the leaf-tips were cut off sufficiently early after the application of the bicarbonate that the normal gradual fall in uptake, characteristic of the behaviour of shoots

in this solution, might reasonably be expected not to have begun. It can be seen in the figure as a slow decline beginning later towards the right-hand end of the graph.

The structure of the leaf-tip hydathodes in relation to the secretion of water

From an examination of the structure of the hydathodes, the removal of which produced the experimental results described, it is not clear by what means they could influence the uptake of water in this way. As Haberlandt (1914) and von Minden found in other plants, there appears to be in *R. fluitans* a continuity of intercellular spaces between the vessel endings in the hydathodes and the external surface of the leaf-tip. On anatomical grounds, therefore, the escape of water from such hydathodes would appear to be merely a filtration process dependent on a positive pressure in the xylem; von Minden and other workers have, in fact, shown that in various other species (mostly of aerial plants) the action of epithem hydathodes is of this nature.

It was found impracticable to repeat observations such as those of Weinrowsky and von Minden on the escape of water from hydathodes in respect of *R. fluitans* in its normal rooted condition. Some observations were, however, made in the laboratory of cut shoots sealed to a glass tube connected in turn to a mercury column and thus subjected to an artificial 'root' pressure.

Under a pressure of approximately one atmosphere (which was the maximum pressure to be withstood by the cycle-valve tubing joint between the plant and the glass tube) no exudation of droplets from the leaf-tips could be seen when these were raised above the surface of the water and examined with a lens. If, however, a leaf was cut off near its base exudation was clearly visible, and if the stems of shoots 25–30 cm. long were cut near the apex, minute droplets were seen to appear at the vascular bundles, and when these were blotted off they reappeared; the water was, in fact, coming through the vascular system and not through the intercellular space system (by injection of the latter under pressure), as might have been the case. That no exudation could be seen from the tips of uninjured leaves was not unexpected when it was borne in mind that each leaf may have over a hundred tips, and the flow from these individually, if any, must have been very small indeed.

When 2 per cent. eosin solution was applied to cut shoots under pressure in the same way, its almost instantaneous penetration of the pith cavity at the lower end of the shoot could be seen, and within a few minutes extensive penetration of the vascular system was also evident, the bundles appearing as fine red lines just below the surface of the stem. If the water surrounding the shoots was kept quite still a diffuse pink halo appeared, after 15–20 minutes, about the leaf-tips of the youngest visible leaf (as yet only just unfolding) and sometimes also round those of the next oldest leaf. Others, however, never showed it unless their tips were cut off, when the dye appeared within a few seconds. It thus appears that the blocking of the epithem (see p. 95) may be complete, or almost so, at an early stage before it becomes evident from normal anatomical examination, so that any outflow from the

vascular system is virtually restricted to those leaves which are still growing. Elsewhere any escape of dye was a result of injury.

Where the experimental shoots bore adventitious roots these became stained except at the tips, but no dye escaped unless the tips were cut off, when it appeared at once. Here, as in the experiments of Priestley and Tupper-Carey (1922), the root-tips readily withstood a hydrostatic pressure sufficient to force water through the vascular system of the shoot.

Although, in these experiments with cut shoots, the pith cavity of the lower part of the shoot became injected with liquid, the diaphragms at the nodes were not readily penetrated, and the intercellular space system was not completely injected. The air in it was, of course, also subjected, to some extent at least, to the applied pressure, but wherever it escaped as face bubbles some injury to the plant was evident. Bubbles did not, for instance, appear from the leaf-tips, in spite of the intercellular space system through the epithem.

Though the natural flow of water from the epithem hydathodes of many aerial plants appears to be a passive filtration process, as is indicated by the experiments of von Minden, and others cited by Burgerstein (1904), Haberlandt remarked on the large nuclei of the epithem cells, which suggested some active function. It is noteworthy that the intercellular spaces of the epithem are always filled with water, in contrast to the air-filled intercellular spaces of the mesophyll. Haberlandt therefore postulated that the function of the epithem, if not that of active secretion of water from the xylem to the exterior, is to keep the intercellular spaces water-filled, so preventing the entry of air into the xylem system when the contents of the latter are under reduced pressure. That the epithem may be of value in this respect in an aerial plant, where the occurrence of negative pressure in the vascular system is normal, is not improbable, but in a submerged aquatic it would appear to be of little importance, and in many aquatics possessing epithem hydathodes the bundle-endings break down quite early in the development of the leaf, leaving the vessels in direct communication with the exterior (Burgerstein, Weinrowsky). Even where the epithem remains, the secretion of water by the epithem cells into their intercellular spaces in the manner envisaged by Haberlandt, this water being drawn from the surrounding mesophyll or from the xylem, would not thereby produce a general flow throughout the xylem system. It is perhaps worth noting that in plants which develop entirely under water the occurrence of water-filled intercellular spaces is, from the physical point of view, far less remarkable than the normal air-filled space system which is a characteristically special feature of aquatic plants.

The virtual blockage of the hydathodes of most leaves on a shoot revealed by the injection of eosin suggests an alternative explanation to the obvious one of the effect of leaf-tip removal on water uptake by the shoot.

Whether the absorbed water was passed to the exterior or not, the hydathodes were clearly not concerned; the outward flow from the shoot, if it occurred, must have been due to the activity of other tissues functioning perhaps by virtue of a polarity with respect to their permeability to water. The

removal of the hydathodes then affected the apparent uptake only because, by opening the vascular system of the shoot, it allowed the influx of water by paths other than the one connected to the potometer. The mechanism whereby, in bicarbonate solution, water should enter the leaves in this way, only to be lost apparently from the tissues elsewhere, raises other problems relating to the internal economy of the shoot.

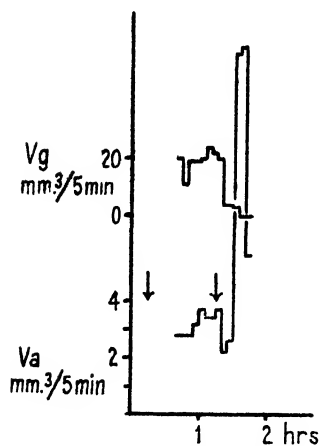


FIG. 12. Uptake by shoot in 0.2 per cent. bicarbonate (1st arrow), and on addition of sodium cyanide to a final concentration of M/5,000 (2nd arrow). The apparent increase in water uptake is due to cessation of gas production.

In *Potamogeton lucens* Thoday and Sykes found that removal of the leaf and tips (as distinct from removal of the leaves) had no effect on the rate of uptake of eosin solution. This result is not inconsistent with the observations on *R. fluitans* described above, if, as Sauvageau stated, the bundle endings in *P. lucens* are in direct communication with the external water, due to an early breakdown of the overlying tissues. In this case, however, there is an anomaly in that it is difficult to see how or why, with their vascular system open to the exterior at every leaf-tip, there should be such a strong 'transpiration tension' in the stems of shoots as was indicated by the rapid uptake of eosin in the experiments of Thoday and Sykes.

The effect of cyanide on the water uptake of cut shoots in bicarbonate solution

The maintenance of a 'transpiration' current in an entirely submerged aquatic plant cannot depend on any purely physical mechanism, as, for example, evaporation. The necessary energy for its continuance must be derived from within the plant, and ultimately from respiration in the living cells. The water uptake observed in these experiments, if not a purely osmotic phenomenon, might therefore be expected to be sensitive to any factor influencing the respiration of the tissues. In view of the established effects of cyanide on respiration, and the reversibility of its influence at high dilutions (Caldwell and Meiklejohn, Emerson, and Genevois), some experiments were carried out to test its effect on the water uptake characteristic of cut shoots in dilute bicarbonate solution.

A preliminary experiment used M/5,000 sodium cyanide. The shoot was set up in the potometer in 0.2 per cent. bicarbonate in the usual way and, when its water uptake had reached a steady value, a weak solution of cyanide was added slowly, over a period of some minutes, to the experimental tank, so as to make a final concentration of M/5,000.

The result of this experiment is shown in Fig. 12. The effect of the cyanide was to produce an almost immediate fall in the absorption rate. There is also another point of interest. The cyanide was sufficiently concentrated

not only to inhibit water uptake, but also to reduce photosynthesis and to stop the evolution of gas from the end of the shoot; corresponding to this the absorption rate showed a transitory increase to a very high value. This was not a true stimulation of the water uptake by the plant, but was due to waterlogging of the pith cavity consequent on the cessation of gas production. It emphasizes that the continuance of the liberation of gas bubbles is essential

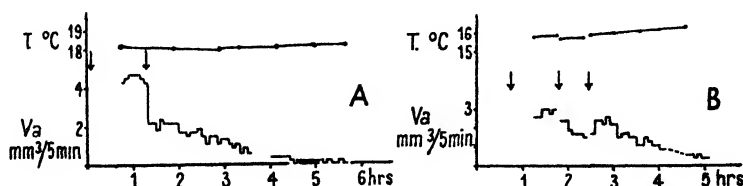


FIG. 13. A, Uptake by shoot in 0.2 per cent. bicarbonate (1st arrow), and on addition of sodium cyanide to a final concentration of M/25,000. (See also Table VIII.) B, Uptake of shoot in 0.2 per cent. bicarbonate (1st arrow), on transfer to 0.2 per cent. bicarbonate plus M/25,000 cyanide (2nd arrow) and return to bicarbonate (3rd arrow). (See also Table IX.)

if measurements of water uptake by volumetric means are to have any real meaning.

At lower concentrations, e.g. M/25,000, cyanide caused a rapid fall in the rate of absorption to about half of its previous value. This new rate remained steady for some time and then began to fall off slowly—the normal decline of absorption in bicarbonate solution (see Fig. 13 A and Table VIII). The reversibility of the response to cyanide at lower concentrations was also established

TABLE VIII

The Effect of M/25,000 Sodium cyanide on Water Uptake of Shoots in 0.2 per cent. Sodium bicarbonate. Uptake is given in mm.³/5 min. for four Periods before and after Addition of Cyanide; one Intermediate Transitional Period has been omitted

Shoot.	(a) Before addition of cyanide.	(b) After addition of cyanide.	Ratio b/a.
1	4.7, 4.7, 4.7, 4.5	2.2, 2.2, 1.9, 2.4	0.47
2	1.7, 1.9, 1.7, 1.9	1.1, 1.1, 0.9, 0.9	0.56
3	1.9, 2.2, 2.2, 2.2	1.1, 0.9, 0.6, 0.9	0.41

by transferring shoots from a tank of bicarbonate solution to a second one of the same concentration of bicarbonate plus cyanide, and back again. A definite recovery of the partially cyanide-inhibited uptake was observed before the normal decline in bicarbonate set in (see Fig. 13 B and Table IX). The results of these experiments indicate a connexion between water uptake and cyanide concentration consistent with the view that the former is affected by the respiratory activity of the plant. In this connexion it should be noted that the tissues of *R. fluitans* contain a direct oxidase system, as shown by the guaiacum test, i.e. an oxidative mechanism of the type known to be inhibited by cyanide.

Some observations were also made of the effect of a low concentration (1 per cent.) of ethyl alcohol on the rate of water uptake. Here a transitory stimulus was detected (see Table X and Fig. 14) similar to that which has been described in connexion with effects of narcotics on the respiration of other

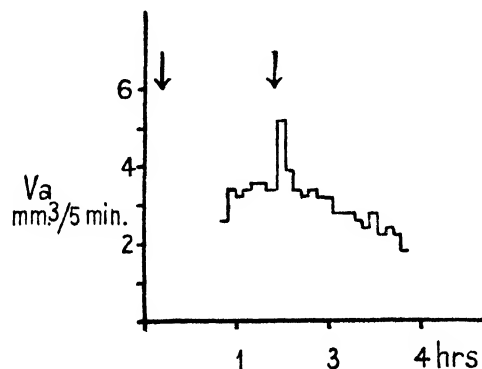


FIG. 14. Uptake by shoot in 0.2 per cent. bicarbonate, and on addition of ethyl alcohol to a concentration of 1 per cent. (2nd arrow). (See also Table X.)

TABLE IX

The Reversible Effect of Sodium cyanide on Water Uptake (mm.³/5 min.) of Shoots in 0.2 per cent. Sodium bicarbonate for three Periods preceding Transfer to the Cyanide, then for Periods while in Contact with the Cyanide, and finally for Periods after Return to the first Solution (see also Fig. 13 B)

	M/25,000 NaCN.				M/37,500 NaCN.				M/50,000 NaCN.				M/75,000 NaCN.		
	(1)	(2)	(3)		(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)
0.2 per cent. NaHCO ₃	3.4	3.0	4.6		3.9	3.9	2.4	3.2	3.7	2.8	4.6	6.0	2.6	2.2	1.7
	3.2	2.8	4.8		3.9	3.4	2.6	2.8	3.4	2.8	4.4	6.4	2.4	2.4	1.7
	3.2	3.0	4.8		3.9	3.9	2.6	3.0	3.7	3.0	4.6	6.2	2.4	2.2	1.7
Means	3.3	2.9	4.7		3.9	3.7	2.5	3.0	3.6	2.9	4.5	6.2	2.5	2.3	1.7
0.2 per cent. NaHCO ₃ + NaCN	2.4	2.4	3.6		2.8	3.0	1.9	3.0	3.0	2.4	4.0	4.4	2.2	1.9	1.5
	2.2	2.4	3.0		2.6	2.4	1.5	1.9	3.0	2.2	3.4	3.6	2.2	1.9	1.1
	1.7	1.9	2.2		2.6	2.2	1.7	1.5	2.6	1.9	2.4	3.4	2.2	1.7	1.3
	1.3	1.7	2.4		2.2	1.9	1.3	1.5	2.4	1.7	2.4	3.0	2.4	1.5	1.3
	1.3	1.7	2.2		2.4	2.2	1.3	1.5	2.4	1.7	2.4	3.2	1.9	1.3	1.1
	1.3	1.5	2.2		2.4	—	—	—	—	1.7	2.4	—	—	1.5	1.1
Means (except two transitional periods)	1.4	1.7	2.3		2.4	2.1	1.4	1.5	2.5	1.7	2.4	3.2	2.2	1.5	1.2
Ratio of mean to original rate	0.42	0.59	0.49		0.62	0.57	0.56	0.50	0.69	0.59	0.56	0.52	0.88	0.65	0.71
	(0.50)				(0.56)				(0.59)				(0.75)		
0.2 per cent. NaHCO ₃	1.3	1.7	2.4		3.2	2.8	1.7	1.7	2.8	2.2	3.8	3.6	2.6	1.5	1.3
	2.2	2.4	3.2		3.4	2.6	1.9	2.2	3.2	2.4	4.6	5.0	2.4	1.9	1.5
	1.7	2.4	3.4		3.2	2.6	1.7	2.4	3.4	2.6	4.6	5.2	2.4	1.9	1.7
	1.7	2.2	3.4		3.4	1.9	1.7	1.9	3.4	2.6	—	5.8	2.4	1.9	1.7
	1.7	2.6	3.2		3.0	1.9	1.9	1.9	3.2	2.4	4.6	5.8	2.2	1.7	1.9
	1.5	2.2	3.0		2.8	2.2	2.2	1.7	—	2.2	4.6	5.6	—	1.7	1.7
	1.1	2.2	2.8		2.4	2.2	1.7	1.5	—	2.2	5.0	5.0	—	1.5	1.5
	1.3	1.5	2.8		2.4	1.7	1.7	1.5	—	—	4.4	—	—	—	—
	—	1.7	—		2.6	1.5	1.7	—	—	—	4.2	—	—	—	—
	—	1.7	—		—	1.5	1.5	—	—	—	4.4	—	—	—	—
Mean of four periods (except one transitional)	1.9	2.4	3.3		3.3	2.3	1.8	2.1	3.4	2.5	4.6	5.5	2.4	1.9	1.7
Ratio of mean to original rate	0.58	0.83	0.70		0.85	0.62	0.72	0.70	0.94	0.86	1.00	0.88	0.96	0.83	1.00
	(0.70)				(0.72)				(0.92)				(0.93)		

plants, e.g. Haas (1919). However, the stimulus of water uptake may have been a largely osmotic one, comparable to that obtained with other solutes (and transitory only on account of the high permeability of plant cells to alcohol) rather than an indirect result of partial narcosis.

TABLE X

The Effect of 1 per cent. Ethyl Alcohol on Water Uptake (mm.³/5 min.) of Shoots in 0.2 per cent. Sodium bicarbonate for Periods preceding and following Transfer from 0.2 per cent. NaHCO₃ to 0.2 per cent. NaHCO₃ plus 1 per cent. Alcohol (see also Fig. 14)

	Shoot.				
	1.	2.	3.	4.	5.
0.2 per cent. NaHCO ₃ .	4.8	5.5	6.0	5.2	3.4
	4.8	6.0	6.4	5.6	3.7
	5.0	6.2	6.4	5.1	3.7
	4.8	6.1	6.2	5.6	3.4
Means	4.9	5.9	6.3	5.5	3.6
0.2 per cent. NaHCO ₃ +	6.0	7.2	7.8	7.6	5.2
1 per cent. EtOH .	5.8	6.4	7.8	6.6	3.9
	5.2	6.7	6.6	6.0	3.4
	5.6	6.8	6.4	6.4	3.2
	5.4	6.7	6.0	5.8	3.4
	5.2	6.8	6.8	6.2	3.2
	5.0	—	6.2	5.8	3.2
	5.0	6.1	5.8	5.4	—
	5.2	6.0	5.8	5.4	—
	5.0	6.0	5.6	5.4	—
	5.2	6.0	5.6	5.6	—
	5.0	5.7	5.2	5.4	—
	4.8	5.7	5.2	5.4	—
	4.4	5.5	—	5.0	—
	4.0	5.5	—	4.6	—
	4.2	5.1	—	6.2	—
	4.2	5.0	—	4.0	—

DISCUSSION

Some experimental results have been described which might be interpreted as indicative of the existence of an upward flow of water in cut shoots of *R. fluitans*, analogous to the transpiration stream of aerial plants. The upward flow appears, however, to be a phenomenon of relatively short duration, and a critical examination of the data must lead to a less positive conclusion.

Attention has been drawn to the apparent absence of any structural feature of the shoot by which the flow might be maintained; in particular the leaf-tip hydathodes appear to be quite inadequate in this role. This is not, of course, in itself a valid argument against the presence of a transpiration stream; it is possible, for instance, that there is a widespread polarity with respect to water in the tissues of the shoot, resulting in all or some of the cells absorbing water on their inner surfaces (ultimately from the xylem) and secreting it externally from their outer surfaces, but until such a 'transpiration' mechanism is established the available data can be interpreted otherwise.

It has been shown that, under the conditions of the potometer experiments, an artificial air-flow (of the same order of magnitude as that produced in a cut shoot by photosynthesis) may, when passed through the intercellular space system of a short length of stem, cause an expulsion of water at an appreciable rate. Though this effect declines in a few hours, in an actively assimilating leafy shoot it may well be more marked and of longer duration. The observed rates of uptake of water by cut shoots are low, variable, not of long duration, and of the same order as the rates of this expulsion of water from the intercellular space system. Such a low rate of flow can hardly be regarded as comparable with the transpiration current of aerial plants, even when the difference in their respective *milieux* is considered, and its physiological significance would appear to be problematical. Though the writer regards rates of uptake of this order as having been critically established under the conditions of the experiments, it is nevertheless doubtful how far this flow represents a real, long-maintained 'transpiration' current through the vascular system of the shoot.

The observed effect of solutes on the water uptake of cut shoots suggests an alternative point of view. It has been shown that various solutes may, in comparatively low concentrations, produce greatly accelerated rates of uptake of water for short periods, and that in general the lower the concentration of the solution the less marked, but more prolonged, is the reaction of the plant. Furthermore, water uptake so induced is reversibly inhibited by cyanide in very low concentrations. Whilst the significance and mode of operation of this solute stimulus have not been elucidated, it is clear that cut shoots exhibit in their water relations a very sensitive balance with respect to external conditions, and possibly also with respect to internal metabolic processes. It is not unlikely, therefore, that in the transport of shoots from a natural environment to the laboratory and in their preparation for experimental treatment, this balance is upset, and water uptake over a limited period may so be initiated. Investigations of the type described necessarily involved the transfer of plants from one environment to another and the handling and preparation of cut shoots. When the experiments were carried out these changes were, perhaps, not fully appreciated, but since the rates of water uptake in tap-water which withstand critical examination are so low, their implications may be considered anew. Such variables as temperature and composition of the water bathing the plant, though under control during the experiments, were subject to change previously, and may have influenced the water balance of the tissues. The experimental evidence thus seems best regarded as indicative of an adjustment of the water balance of the tissues of the shoot rather than of a long-maintained flow through the xylem system to the exterior. The uptake observed may, in fact, be analogous to that produced by the stimulus of solutes in the external water, but of lesser intensity and longer duration, and on these lines the experimental data can be harmonized. The negligible uptake of eosin by cut shoots prepared 10–15 hours before use is explained, and the effect on water uptake of the removal of the

leaf-tips is understandable, simply because this operation opens up the xylem system to the exterior.

The writer regards other reported instances of uptake of water by cut shoots as being open to a similar interpretation. The experiments of Thoday and Sykes, indicating a very rapid water movement in the xylem, were all of very short duration, carried out on plants shortly after cutting; this operation may have caused unappreciated disturbances in their internal balance. Similar experiments, in some instances with the same species, of Thut, where the absorption was observed over a period of some hours, led him to conclude that the leaves were not, as Thoday and Sykes supposed, significant contributors to the water flow. The experimental conditions of both these investigations appear to have been such that changes in temperature and other external factors can hardly have been operating, and the extreme rapidity of the uptake observed by Thoday and Sykes is suggestive of the presence in the xylem lacunae of air or water vapour under reduced pressure, though how this condition would arise is admittedly obscure.

Escape of water from the hydathodes, if it occurs, is therefore to be regarded as a passive filtration process, as in fact the older workers maintained, taking place only under conditions of positive pressure in the xylem as a result of an active root system and not as a transpiration current (in so far as this term implies action in the leaves). That this is so in aerial plants seems well established, and the similarity in structure of the hydathodes of a submerged aquatic such as *R. fluitans* to those of aerial plants points to a similar conclusion. No difficulties then arise as to the mechanism which would, on the 'transpiration' hypothesis, have to be attributed to the hydathodes in relation to the exudation. In aerial plants the epithem is probably of far less importance in its influence on the outflow of water than in preventing the ingress of air into the xylem system. The epithem in submerged aquatics is thus to be regarded as a relic of little or no physiological importance, and its early disappearance in the development of the leaves of many such plants becomes intelligible.

The presence of a true 'transpiration' current, i.e. an active flow of water in the shoot by forces residing in the leaves, has thus not been established. A long-maintained flow in the xylem, if it occurs, must be due to root pressure, or possibly, since the endodermis forms so complete a sheath to the vascular system, to exudation pressures existing elsewhere in the shoot.¹ It has been shown that a pressure of one atmosphere may produce a readily observable mass flow of water in the xylem system of cut shoots of *R. fluitans*, but the presence of such a natural flow in intact plants has not been investigated. Experiments by Thut on this point have been critically considered; they indicate the presence of a root pressure in his plants, but quantitatively they are less satisfactory. The marked decline in the rate of water movement with

¹ It has been suggested by Priestley (1932) that exudation pressure may be not only a property of the root, but may also arise in tissues of the shoot. Thut, however, rejects this view on experimental grounds in relation to the aquatics with which he worked.

time, both in complete plants and in rooted stumps, is of unknown significance; it recalls the transient uptake observed by the writer, though in these experiments of Thut where root pressure was involved, oxygen deficiency may have been the causal factor. His whole plants were enclosed in relatively small vessels, and his rooted stumps, though in their natural environment, may have been deprived of oxygen in their lower parts by the opening up of the intercellular space system allowing the escape of photosynthetic oxygen.

The behaviour of cut shoots of *R. fluitans* in dilute solutions, which has been described, raises other problems. Though the observed stimulation of water uptake would apparently admit to some extent of an explanation on osmotic grounds, the behaviour of the plant in different solutions, and the after effects, point to other physiological complications. In particular, the reversible depression of the solute-stimulated uptake by very dilute cyanide suggests that the water relations of the tissues are not purely osmotic, but may be linked, in some way as yet unelucidated, with other properties and functions of the living cell.

SUMMARY

The evidence bearing on the 'transpiration' stream in submerged aquatic plants is reviewed.

Experiments, similar to those of earlier workers, on the uptake of water by cut shoots of *R. fluitans* are described, using the ascent of eosin in the vascular system as a means of measurement. Though uptake was observed, it appears to be a relatively short-term phenomenon, detectable in freshly collected material, but dying away in 10–15 hours.

The special conditions, arising from the large intercellular spaces of aquatics, which must be complied with if accurate volumetric measurement of water uptake by these plants is to be made, are discussed; and a potometer is described which meets these conditions as far as possible. Using this potometer, it was found that cut shoots of *R. fluitans* might show uptake of water at rates initially of the order of 1–10 mm.³ per hour, but decreasing markedly in a few hours. In addition, exudation of water also commonly occurred and in other instances no water movement could be detected. It is shown that the passage of a slow air stream, such as might arise from the photosynthetic activity of the shoot, through the intercellular spaces may commonly displace water from them at rates initially of the same order as the observed rates of uptake. The physiological significance of this uptake, in comparison with the transpiration stream of aerial plants, is thus called into question.

The structure of the epithem hydathodes of *R. fluitans* is discussed in relation to water movement. Like similar structures of aerial plants, these appear to be entirely passive in action. No observations of natural exudation from them have been made, but artificially applied 'root' pressures of the order of one atmosphere produce a readily observable mass flow through the vascular system of the shoot and the hydathodes of the youngest leaves; in older leaves the hydathodes become occluded.

Stimulatory effects of externally applied solutes on the water uptake of cut shoots are described. Though these appear to be in part osmotic in nature, they present features pointing to the interplay of other physiological properties of the cell. It is evident that cut shoots of *R. fluitans* exhibit a sensitive water balance with respect to the environment, and the observed short-term water uptake of such shoots would appear to be better regarded as an adjustment of this balance than as evidence of a long-maintained 'transpiration' stream. Such a flow, if it occurs in naturally rooted plants, must be attributed to root pressure. This conclusion is in accordance with the structure of the leaf-tip hydathodes, from which, under conditions of positive pressure in the vascular system, the outflow of water may occur.

ACKNOWLEDGEMENTS

It is a pleasure to the writer to acknowledge his indebtedness to Emeritus Professor T. G. Hill, under whose supervision this work was begun at University College, London, for much help and encouragement, and also to Dr. F. C. Steward of Birkbeck College for criticism and advice in its later stages.

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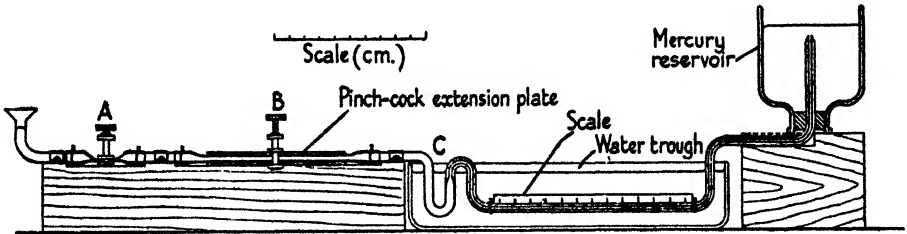
NOTE

A simple form of the Bonnier and Mangin gas-analysis apparatus.—

The Bonnier and Mangin apparatus for the determination of carbon dioxide and oxygen in small volumes of gas mixtures is well known, and a full account of the apparatus and its mode of operation has been given by Thoday (*Ann. Bot.*, xxvii. 565, 1913).

It is not proposed here to discuss the apparatus in detail, but briefly to describe a simple form which has the especial merit, in these days, of being assembled from odds and ends to be found in any laboratory. Nevertheless, if operated in accordance with Thoday's findings, it is sufficiently accurate for many purposes.

The general arrangement is shown in the accompanying figure. The cylinder and screw-operated piston of the original form of the apparatus, by means of which the



gas sample and reagents are drawn into and expelled from the tube, are replaced by a length of rubber tubing compressed by a screw pinch-cock B. The latter has its jaws extended to a length of about 9 cm. by stiff brass plates soldered in position, and so, in compressing the rubber, produces a change in volume sufficiently large for the operation of the apparatus. The other pinch-cock A, and the funnel-shaped opening shown at the left-hand side of the drawing, are used only in filling the tube with mercury, and in operation this pinch-cock is kept closed. The capillary tubing should be selected for regularity of bore—a cross-sectional area of about 2 mm.² is a convenient size—and the horizontal portion, in which measurement of gas volumes is made, is fitted with a transparent mm. scale. This part is immersed in water in a shallow trough to minimize short-period fluctuations in temperature; the bottom of the trough can with advantage be painted white to give maximum legibility of the scale. The bends in the tube at C serve as a trap to prevent the accidental passage of the gas sample into the rubber tubing, where it would be liable to become partially lost.

The apparatus requires about 0.2 c.c. of gas for each sample, so as to give an initial length of column in the tube of about 100 mm., though, of course, smaller quantities can be used with correspondingly larger errors in the final result. The scale can be read by interpolation, with the aid of a lens, to 0.1 mm. so that a theoretical accuracy of 0.1 per cent. of the total volume should be attainable, and in practice it has been found that replicate determinations seldom differ by more than

0.2 per cent. Carbon dioxide in normal air cannot thus be detected, but for analysis of the gas given off by submerged aquatic plants and of the air in contact with respiring tissue the apparatus has proved its worth.

In conclusion, a note of warning: it is impossible for the operator to keep his fingers entirely free from the reagents, and as the alkaline pyrogallol used for oxygen absorption is particularly destructive of the skin, rubber gloves, or at least rubber finger-stalls for one hand, are a necessary part of the apparatus.

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Physiological and Ecological Studies in the Analysis of Plant Environment

II. The Interaction between Light Intensity and Mineral Nutrient Supply in the Growth and Development of the Bluebell (*Scilla non-scripta*)

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With fourteen Figures in the Text

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INTRODUCTION²

THE first paper of this series (Blackman and Rutter, 1946) was concerned with an analysis of the light factor in relation to the distribution of the ground flora in woodland communities. By the design of the experimental techniques, coupled with a special statistical treatment of the data, it was shown that the variations in the density of the bluebell (*Scilla non-scripta*) were highly correlated with variations in the degree of shading. In fact, in the three woodland communities examined in detail no less than up to three-quarters of the total variation in density could be attributed to the light factor. There was also evidence that in deciduous woodland the degree of shading during the high light phase of early spring (March–April) was of greater importance than the low light phase of early summer (June).

Apart from establishing the importance of shading in relation to the distribution of the bluebell, there was indirect evidence, (a) that the bluebell is not

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² Some of the results given in this paper have been included in a thesis submitted by the second author for a degree of Doctor of Philosophy of the University of London.

only intolerant of deep shade but is also sensitive to relatively slight shading, and (b) that at the higher light intensities other factors than light may be operative.

In the present investigation the aim has been to determine experimentally the effects of shading and mineral nutrient supply on seasonal growth and development. The experiments can be divided into two series, one concerned with the growth of bluebells in woodland and the other with bulbs planted in experimental plots where the plants were shaded artificially by means of screens.

EXPERIMENTAL RESULTS

1. *Seasonal growth of the bluebell under woodland conditions*

The experiments on the growth of the bluebell in woodland were considered as a prerequisite to the designing of the field experiments. Preliminary information was needed both to throw light on the magnitude of the seasonal growth under natural conditions and to establish the errors attached to such measurements. The site selected for the first experiment (expt. 1) in 1937 was an open oak (*Quercus robur*) wood at Warfield, Berks., with a thin under-story of coppiced hazel (*Corylus Avellana*). Within the wood six blocks of plots were selected in areas where hazels were absent and the bluebells formed almost pure stands. Each block was divided into four plots, of which three plots received in late February additions of nitrogen, phosphorus, or potassium respectively, while the fourth was untreated. The nitrogen was applied as ammonium nitrate at a rate equivalent to 50 lb. of nitrogen per acre and both the phosphorus (sodium dihydrogen phosphate) and the potassium (potassium sulphate) at a rate equal to 75 lb. per acre.

From the middle of March, when the bluebell shoots first appeared above ground, until the end of May, when the senescent phase had set in, the plots were sampled at weekly intervals. In addition a final sample was taken at the end of July when all the leaves had died back and the seeds shed. The method of sampling was to collect and count all the plants from ten random quadrats (6 in. \times 6 in.) per plot. After washing the roots free from soil the plants from each group of ten quadrats were then divided into root plus bulb and shoot (inflorescence plus green leaves) and the dry weights determined separately.

In addition, during the season the light intensity, relative to full daylight, was measured by the method described in the previous paper—matched photo-electric cells. On each sampling occasion the measurements were made at the corners of each plot, i.e. at 96 points.

The seasonal changes in both the weight of the whole plant and the weight of the shoot are shown in Fig. 1, together with the fall in the light intensity during the period. Since statistical analyses of the data showed that none of the manurial effects was significant, the four manurial treatments have been grouped together. It is seen from Fig. 1 that whereas during March and early April the shoot weight is increasing the whole plant weight shows little change. From mid-April onwards, however, there is a relatively rapid rise in both shoot

and whole plant weight. These reach a maximum at the end of May; subsequently the period of senescence sets in.

During the active period of growth the light intensity fell steadily from 0.84 of daylight before the oaks 'broke' to 0.33 of daylight when the leaf canopy was fully expanded. Over this period the total plant weight was trebled, but comparing the final bulb weight in July with the whole plant weight in March the gain was only some 60 per cent. for the season.

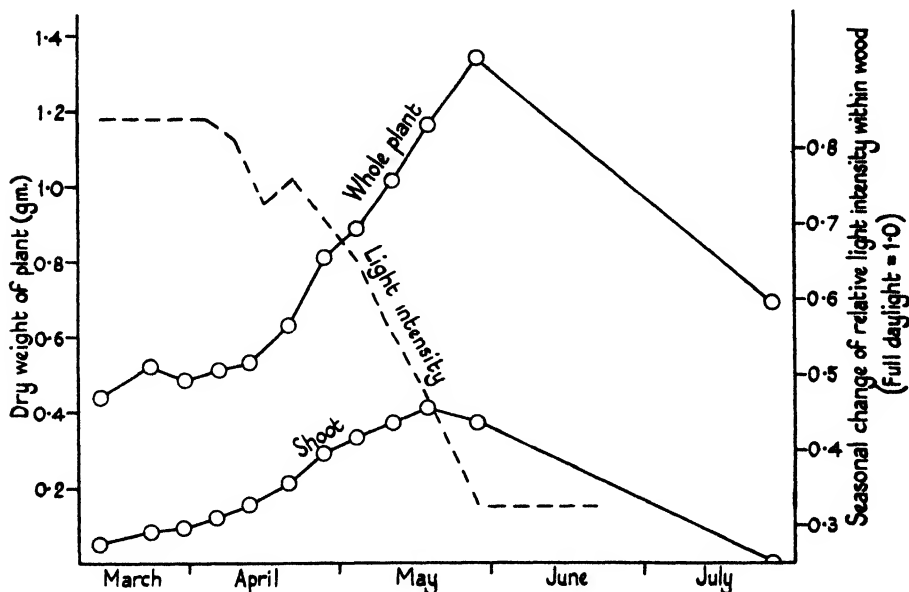


FIG. 1. The seasonal growth of *Scilla non-scripta* in an open oak wood (expt. I, 1937).

Since during the early spring increase in shoot weight is not linked with a gain in total plant weight, transference of food reserves from the bulb to the shoot must have taken place. As the leaves, though not fully expanded, had a considerable assimilating surface, the failure of the plants to gain weight may be ascribed to low temperature limiting assimilation. Moreover, it is likely that during this period of shoot extension the respiration losses are high.

It seemed equally probable that during the autumn and winter respiration losses would also be considerable, since it was observed that as soon as the autumn rains penetrated as far as the dormant bulbs both root development and shoot extension were initiated. In order to gain some estimates of this loss some 360 graded dormant bulbs were divided into 36 sample lots and their fresh weights recorded. Four lots were then taken at random; the percentage water content was determined, and from the average water-content the dry weights of the remaining 32 lots were estimated. These lots were then planted in a hazel coppice at the end of July. Subsequently at monthly intervals 4 samples were dug up, the dry weights determined, and the changes

in weight expressed as a percentage of the initial weight. The results are shown in Fig. 2, and it is clear that there is a steady and uniform fall in weight during the autumn and winter. By the time that the shoot appears above ground in March nearly half the original autumn weight has been lost.

2. *Seasonal growth of the bluebell under varying levels of light intensity and nutrient supply*

The results of experiment I demonstrated that even under conditions of relatively high light intensity, the annual rate of growth in woodland is small.

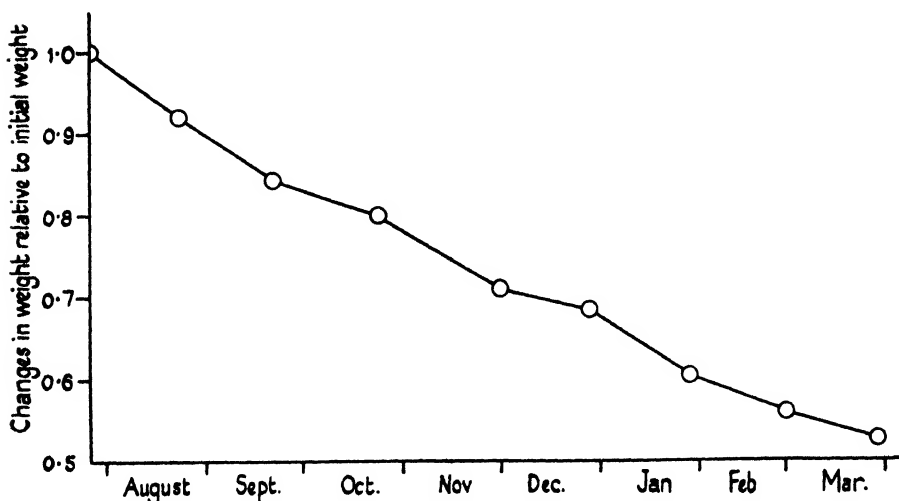


FIG. 2. Loss in weight of individual plants of *S. non-scripta* growing under woodland conditions during the 'dormant' phase, i.e. from root initiation in the late summer to shoot emergence in the early spring (expt. II).

As a result of this slow rate of growth, it was evident that considerable accuracy would be required to detect significant changes at lower light intensities. In woodland it was equally clear that one of the sources of error was the exceedingly large variation in plant and bulb size which showed more than a 500-fold difference between the largest and smallest plants. Consequently to measure accurately the rate of growth over short intervals of time very large samples would be required. Moreover, there was the further disadvantage that the flowering stage is related to bulb size, and therefore the woodland samples consist of a heterogeneous mixture of flowering and non-flowering plants. Finally, it is difficult to relate precisely changes in the growth rate with the degree of shading when the intensity of shading is rising progressively during the season.

In consequence it seemed evident that complementary information could be obtained from field experiments in which bulbs, graded to size, were employed and the degree of shading by means of screens kept constant during the season.

Experimental technique. The experimental design of all the experiments was on similar lines in the three years (1938-40). In each season the effects of either three or four levels of light intensity were investigated. These light treatments were combined with manurial treatment in a multi-factorial arrangement. Bulbs, graded for size by eye, were employed and for individual experiments consisted of either large flowering bulbs or small non-flowering bulbs.

In three out of the four largest experiments on flowering bulbs there were three levels of light intensity combined with eight manurial treatments—no manure (C), nitrogen (N), phosphorus (P), potassium (K), nitrogen and phosphorus (NP), nitrogen and potassium (NK), phosphorus and potassium (PK), and nitrogen, phosphorus, and potassium (NPK)—making in all 24 treatments. Since the replication was twofold there were 48 plots. In order to reduce the errors due to a large block size, the second order interaction between nitrogen, phosphorus, and potassium was confounded. Thus at all light intensities the treatments N, P, K, and NPK were assigned to one block and C, NP, NK, and PK to the other. There were, in consequence, four instead of two blocks. In the fourth experiment there were four levels of light intensity but no manurial treatments.

The plot size had perforce to be small since otherwise the screens used for shading the plots would have been too heavy and unwieldy for periodic removal. Moreover, by keeping the screens small, the indirect effects of shading, especially the raising of the air temperature, would be minimized owing to diffusion and convection currents. In each plot some 45-50 dormant bulbs were planted during the autumn in rows 9 in. apart with 6 in. between each bulb in the row.

The nutrient treatments were added prior to shoot emergence in February or early March. The rates of application and the materials used were the same as in the woodland experiment (expt. I), namely, 50 lb. of nitrogen per acre and 75 lb. each of potassium and phosphorus.

In the several experiments a preliminary sample was taken when the plants were first shaded in April. Intermediate samples were taken during the growing season and a final sample when the seeds had been shed and the bulbs were dormant. Samples consisted normally of 4 to 5 plants per plot. In 1939 the sample number on some plots had to be reduced owing to rejection of bulbs attacked by a *Penicillium* and subsequently identified by Singh (1941) as *P. cyclospium*.

The individual plants were dug up with a narrow trowel and washed to remove soil particles. The plants from each sample were divided into bulb, leaf, and inflorescence before being weighed and were subsequently dried at 100 C° in an oven with a forced draught. For the purpose of separation, the term 'leaf' implies all the green assimilating area but does not include the basal parts below ground level.

In order to reduce the light intensity, a wooden frame covered with one of several materials was placed on the appropriate plot. Each screen was supported on wooden corner-posts some 12 inches off the ground and

the screen extended over the whole plot. In 1938 an open black gauze and some black 'lawn' were employed to reduce the light intensity, but periodic measurements with the matched photo-electric cells showed that the degree of shading altered with time, due to the fading of the black dye and the washing out of the 'filler' from the threads of the fabrics.

To avoid this source of error the black gauze and lawn were discarded in subsequent years and shading controlled by using either white butter-muslin or perforated zinc sheets covered with a thin film of vaseline to minimize both the risk of corrosion and the solution of the zinc in the rain passing through the screens. Since with the zinc sheets the percentage of light transmitted depends on the ratio of the areas of metal surface to perforations, there was no question of any variation in light transmission. Tests on the butter-muslin showed that there was no appreciable change in light transmission during the experimental period.

In order to standardize the time when the screens were first to be placed on the plots in the spring, apart from observation of the bluebell plants, a phenological criterion was also taken into account. Previous observations had indicated that the active growth phase of the bluebell coincided with the 'breaking' of larches in the neighbourhood and this occurred in 1938-39-40 on April 5, 12, and 24 respectively.

Statistical treatment of the data. Since the growth of the bluebell until mid-season is approximately exponential, the error at any time is roughly proportional to the square of the mean weight. This results in an unequal distribution of error, and this inequality may lessen the sensitivity of the analysis of variance of the data over all occasions. The *L* 1 test of Nayer (1936) was therefore applied to the data to determine whether the ratio of the geometric mean of the error variances to the corresponding arithmetic mean departed from unity. From Table I it is seen that for changes in plant weight with occasion, the ratio is significantly less than unity. However, if a logarithmic transformation of the weight data is employed, then there is no longer any significant departure.

TABLE I

*Effect of Light Intensity and Nutrient Supply on Seasonal
Changes in Plant Weight*

Values of Nayer's L 1 Test for Sample Errors on Untransformed and Logarithmic Scales

	L 1.		L 1 required.	
	Untransformed scale.	Logarithmic scale.	5%	1%
Experiment III	0.707	0.912	0.898	0.848
„ IV	0.849	0.933	0.903	0.867

In consequence of the necessity to apply this logarithmic transformation, significant differences calculated from the analysis of variance can only be applied to the means of the logarithms, and, where necessary, both the transformed and untransformed data have been included in the tables. The use of the logarithmic scale for growth data has, however, the advantage that it provides a better test of the independence or interaction of treatments (Fisher and Mackenzie, 1933, and Cochran, 1938).

Effects of light intensity and nutrient level on the seasonal growth of mature flowering bulbs

The influence of light intensity, coupled with nutrient supply, on the growth of flowering bulbs was investigated at Imperial College Biological Field Station, Slough, for three seasons, 1938-40.

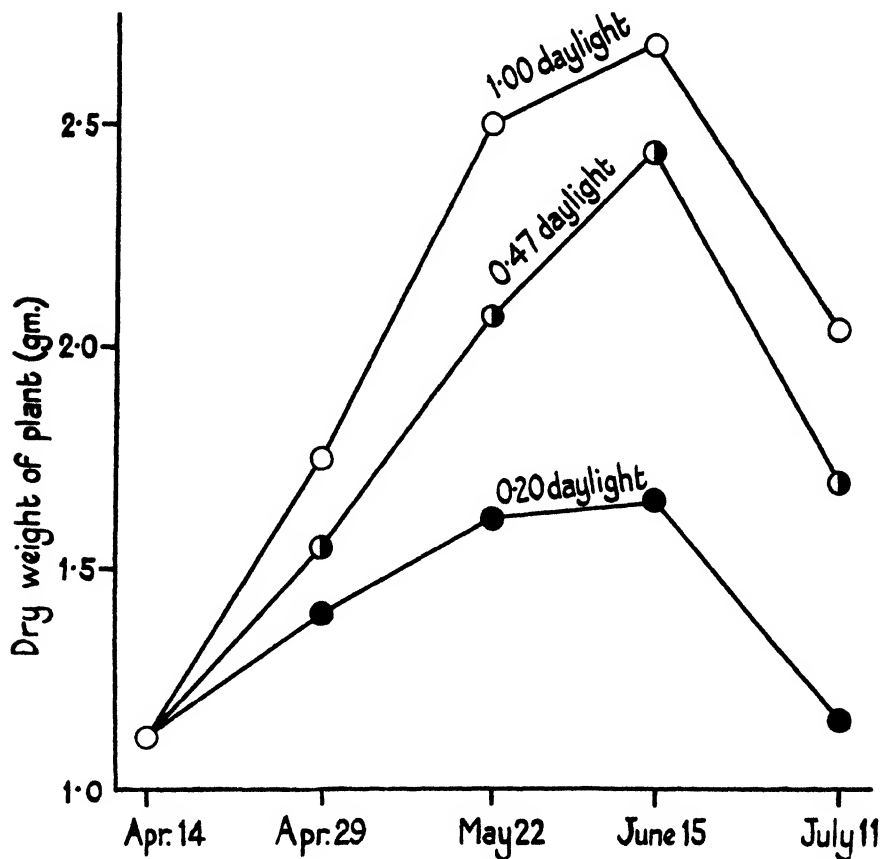


FIG. 3. The effects of varying light intensity on the seasonal growth of flowering plants of *S. non-scripta* (expt. III, 1938).

In the first of the experiments it has already been pointed out that under the action of rain and sun the screening materials faded. In consequence there was gradual increase of light transmission during the season, especially during June (see Table II).

The effects of the varying mean light intensity on the changes in the weight of the whole plant and of the bulb, leaf, and flower are given in Figs. 3-6. Taking first the whole plant data (Fig. 3) it is at once evident that shading has a marked and progressive effect. Even with the relatively slight shading of 0.47 daylight (L 2) there is an appreciable decrease in growth. That this

depression is highly significant is evident from Table III where both the data, transformed on a logarithmic scale for statistical treatment, and the actual data are included. The statistical analysis also shows that there is a significant interaction between light intensity and occasion—that is, that the

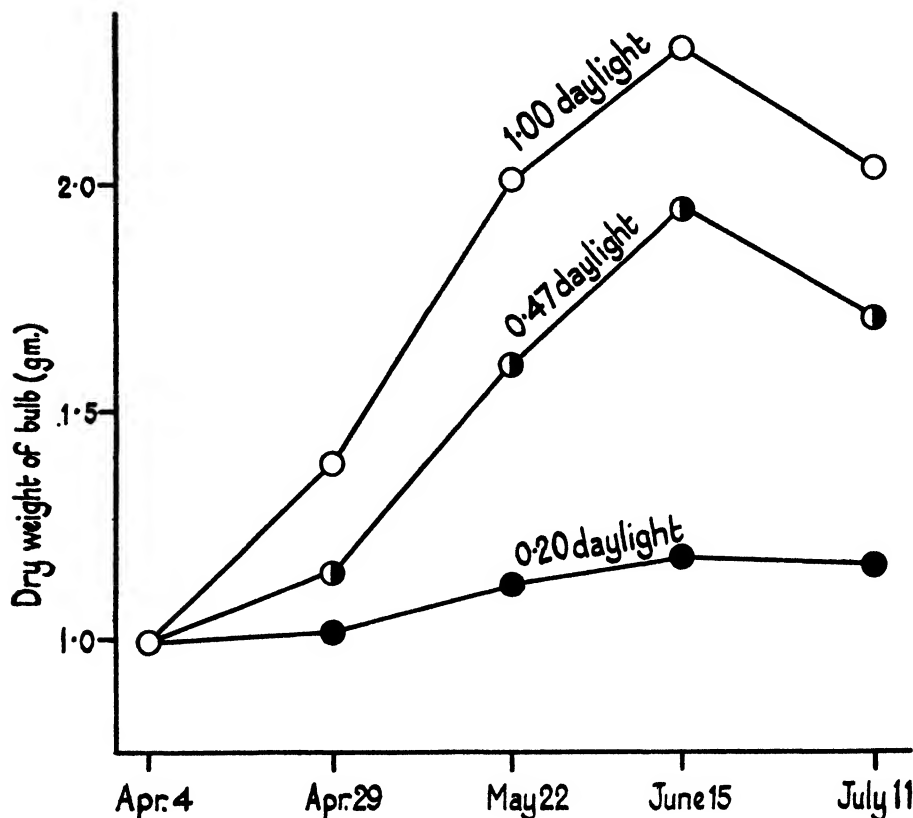


FIG. 4. The effects of varying light intensity on the seasonal changes in bulb weight of *S. non-scripta* (expt. III, 1938).

TABLE II

Effects of Exposure to Weather on Light Transmission through Gauze and Lawn Screens

Experiment III.

Date of observation.	Light intensity under screens relative to full daylight.	
	Black gauze.	Black lawn.
April 19	0.395	0.181
May 6	0.440	0.211
May 24	0.445	0.171
June 3	0.542	0.196
June 21	0.602	0.288
Mean	0.465	0.201

light effects are progressively increased until the last sampling occasion, that is after the seeds had been shed and the bulb had become dormant.

The changes in the bulb weight—seen in Fig. 4—follow closely the changes in total plant weight. This is not surprising since the bulb forms a large proportion of the total plant weight—see Table III. It is noteworthy, too, that

TABLE III
The Effects of Light Intensity on the Seasonal Growth of Mature Flowering Plants

Experiment III, 1938

Light intensity.		Dry weight (gm.) per plant.					All occasions.
		Sampling date.					
		April 4.	April 29.	May 22.	June 15.	July 11.	
1.0 daylight (L 1)	Bulb	0.874	1.381	2.036	2.300	2.041	—
	Whole plant	1.093	1.753	2.505	2.682	2.041	2.015
	Whole plant,*	0.736	0.940	1.086	1.120	1.005	0.977
	log. scale						
0.47 daylight (L 2)	Bulb	0.912	1.144	1.601	1.943	1.697	—
	Whole plant	1.125	1.555	2.071	2.443	1.697	1.778
	Whole plant,	0.746	0.887	1.012	1.080	0.933	0.932
	log. scale						
0.20 daylight (L 3)	Bulb	0.913	1.013	1.132	1.177	1.160	—
	Whole plant	1.136	1.400	1.617	1.659	1.160	1.394
	Whole plant,	0.751	0.839	0.900	0.913	0.762	0.833
	log. scale						
All light intensities	Bulb	0.900	1.179	1.590	1.807	—	—
	Whole plant	1.118	1.569	2.065	2.262	1.633	—
	Whole plant,	0.744	0.889	0.999	1.038	0.900	—
	log. scale						
Whole plant (log. scale).							
Significant difference between light intensity means						= 0.016	
"	"	"	occasion means			= 0.013	
"	"	"	light × occasion means			= 0.030	

* Log. weight of 5 plants.

at the lowest light intensity the gain in bulb weight over the growing period is very small. In fact, considering that the weight of the individual bulbs when planted in the previous autumn was 1.060 gm., the gains in weight over the full annual cycle were 94, 60, and 9 per cent. for full daylight, half daylight, and one fifth daylight.

It is not unexpected that the effects of the light factor on the development of flowers and leaves are relatively small (Figs. 5 and 6), since the leaf and flower primordia are laid down in the previous autumn. In the case of the leaves (Fig. 5) the only statistically significant effect is on the last occasion, where the smaller leaf weight of the unshaded plants can be accounted for by the observed earlier onset of senescence.

Apart from the main light effects there were a number of less-marked manurial effects. Over the whole season there was a slight but significant

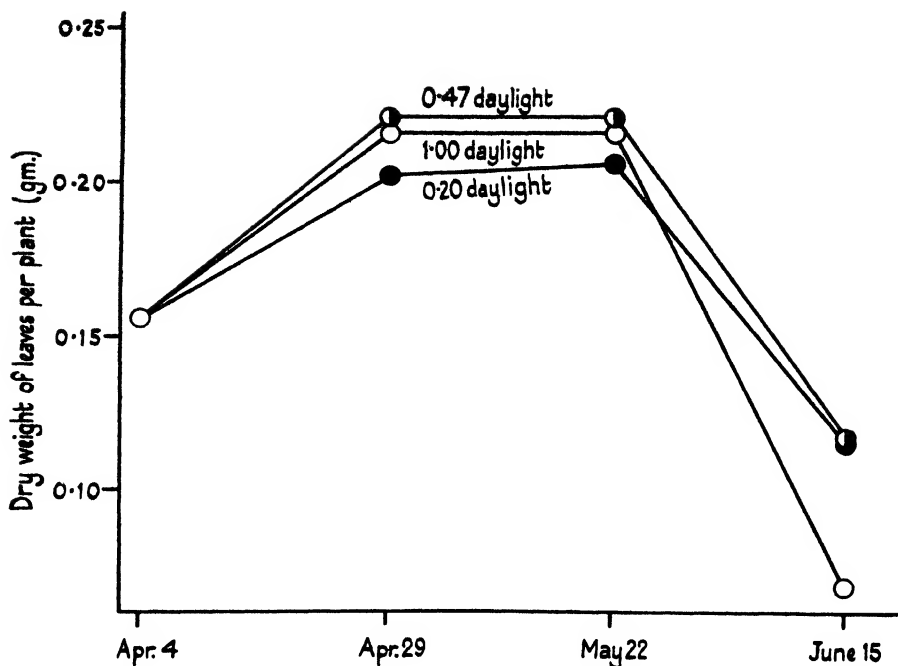


FIG. 5. The effects of varying light intensity on the seasonal changes in leaf weight of *S. non-scripta* (expt. III, 1938).

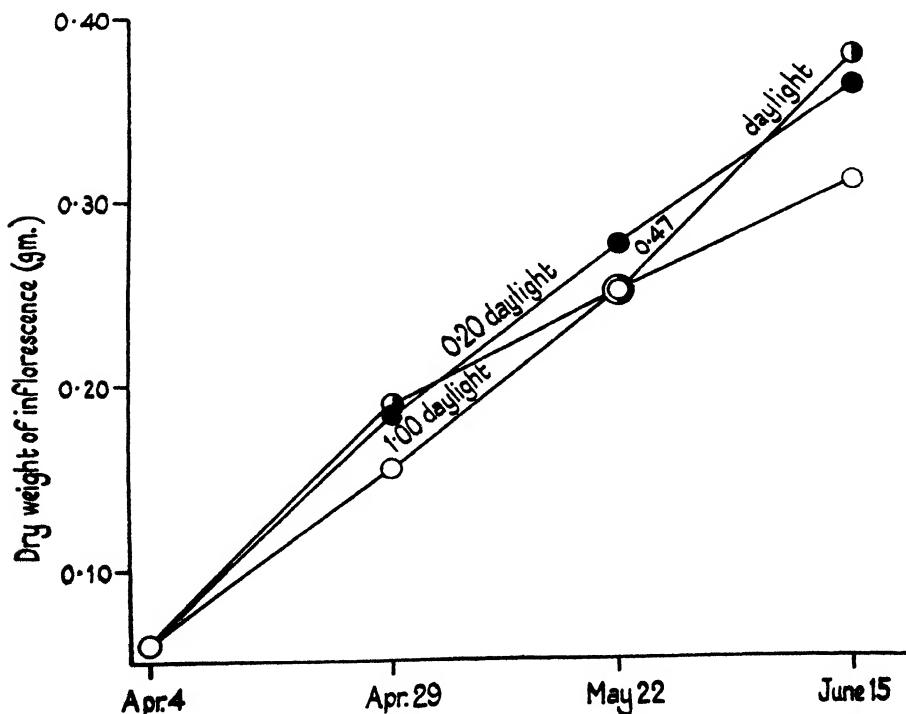


FIG. 6. The effects of varying light intensity on the seasonal changes in inflorescence weight of *S. non-scripta* (expt. III, 1938).

increase in total plant weight due to additional nitrogen but this effect had no significant interaction with either light intensity or sampling occasion. From Table IV it is also seen that neither phosphorus nor potassium caused any change.

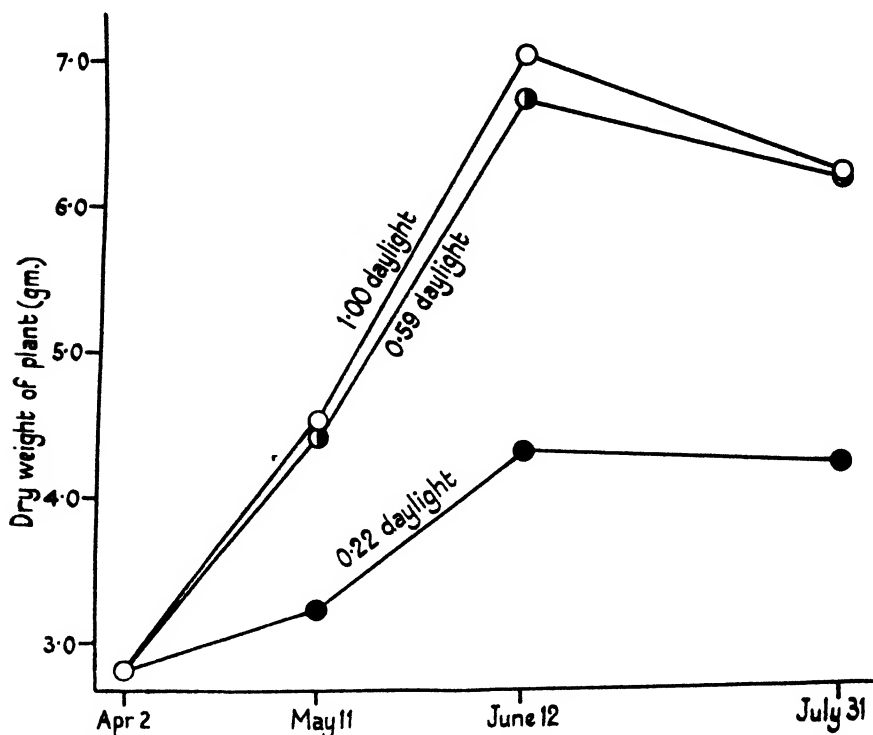


FIG. 7. The effects of varying light intensity on the seasonal growth of flowering plants of *S. non-scripta* (expt. IV, 1939).

TABLE IV

The Effects of additional Nitrogen, Phosphorus, and Potassium on the Development of Flowering Plants

Experiment III, 1938

Mean dry weight (gm.) per plant over all samples.

	Nitrogen effect.		Phosphorus effect.		Potassium effect.	
	N.	No N.	P.	No P.	K.	No K.
Bulb	1.395	1.334	1.357	1.372	1.385	1.344
Leaf	0.179	0.165	0.169	0.175	0.175	0.169
Flower	0.206	0.218	0.222	0.203	0.208	0.217
Whole plant	1.761	1.690	1.719	1.732	1.742	1.709
Whole plant, log. scale	0.924	0.904	0.912	0.916	0.917	0.911

Whole plant, log. scale; sig. diff. ($P = 0.05$) = 0.012

In the 1939 experiment (expt. IV) large flowering bulbs were employed and the changes in seasonal growth due to varying light intensity are seen in Fig. 7 and Table V. It is again found that in full daylight the bulb weight

is doubled over the course of the annual growth cycle, i.e. a bulb weight of 3.36 gm. at planting in the autumn has become 6.31 gm. on July 31, when the bulbs were again dormant. A reduction in the light intensity to 0.59 of daylight causes no corresponding decrease in the whole plant weight, but a further reduction to 0.22 daylight leads to a decrease in plant size. From Table V it is also evident that as in the previous year changes in bulb weight are closely linked with changes in total plant weight.

TABLE V
*The Effects of Light Intensity on the Seasonal Growth of
Flowering Plants*

Experiment IV, 1939

Dry weight (gm.) per plant.

Light intensity.		Sampling dates.				All occasions.
		I. April 12.	II. May 11.	III. June 12.	IV. July 31.	
1.0 daylight (L 1)	Bulb	2.114	3.450	5.839	6.310	—
	Leaf	0.342	0.726	0.466	—	—
	Inflorescence	—	0.366	0.769	—	—
	Whole plant	2.456	4.542	7.074	6.310	5.975
	Whole plant, log. scale	—	—	—	—	0.763
0.59 daylight (L 2)	Bulb	2.106	3.112	5.687	6.281	—
	Leaf	0.341	0.808	0.481	—	—
	Inflorescence	—	0.490	0.614	—	—
	Whole plant	2.447	4.410	6.782	6.281	5.824
	Whole plant, log. scale	—	—	—	—	0.755
0.22 daylight (L 3)	Bulb	2.136	1.834	2.981	3.999	—
	Leaf	0.359	0.753	0.601	—	—
	Inflorescence	—	0.310	0.441	—	—
	Whole plant	2.495	2.897	4.023	3.999	3.640
	Whole plant, log. scale	—	—	—	—	0.552
All light intensities	Bulb	2.118	2.799	4.836	5.530	—
	Leaf	0.347	0.762	0.516	—	—
	Inflorescence	—	0.389	0.608	—	—
	Whole plant	2.465	3.950	5.960	5.530	—
	Whole plant, log. scale	—	0.585	0.758	0.726	—

Flower included with bulb in sample 1.

Whole plant (log. scale).

Significant difference ($P = 0.05$) between light intensity means = 0.030

" " " " occasion means = 0.026

No significant interaction

In contrast to the 1938 results there were a number of marked effects of nutrient supply. Over all occasions, additional nitrogen, phosphorus, and potassium have resulted in significant increases in plant weight (Table VI). Moreover, these manurial effects are dependent on the level of the light intensity. This interaction between the light and manurial factors is evident

from Fig. 8, where the gains or losses in plant weight relative to the controls have been plotted for each light intensity. For the several manurial factors the greatest contrast is between the highest and lowest light intensities.

TABLE VI

The Effects of Additional Nitrogen, Phosphorus, and Potassium on the Growth of Flowering Plants

Experiment IV, 1939

	Mean dry weight (gm.) over samples II-IV.					
	Nitrogen effect.		Phosphorus effect.		Potassium effect.	
	N.	No N.	P.	No P.	K.	No. K.
Bulb	3.862	3.773	4.024	3.610	3.870	3.766
Whole plant	5.338	4.954	5.362	4.930	5.329	4.964
Whole plant, log. scale . .	0.703	0.676	0.705	0.675	0.702	0.678

Log. scale; significant difference ($P = 0.05$) = 0.025

The significance of these light-nutrient supply interactions for plant weight over sampling occasions II-IV can be judged from Table VII. In the case of both phosphorus and potassium the increase in plant weight in full daylight significantly exceeds the corresponding changes at the lowest light intensity (0.22 of daylight).

TABLE VII

The Interaction between Light Intensity and Mineral Nutrient Supply in the Growth of Flowering Plants

Experiment IV, 1939

Light intensity.		Mean dry weight (gm.) over samples II-IV.					
		Nitrogen effect.		Phosphorus effect.		Potassium effect.	
		N.	No N.	P.	No P.	K.	No K.
1.00 daylight	Whole plant	6.341	5.617	6.451	5.503	6.363	5.590
	Whole plant, log. scale	0.787	0.739	0.795	0.730	0.791	0.735
0.59 daylight	Whole plant	6.003	5.701	6.016	5.688	6.097	5.607
	Whole plant, log. scale	0.766	0.744	0.766	0.743	0.772	0.737
0.22 daylight	Whole plant	3.682	3.598	3.648	3.632	3.542	3.738
	Whole plant, log. scale	0.558	0.546	0.552	0.552	0.543	0.562

Log. scale: significant difference between light intensity \times nutrient supply means = 0.043

Fig. 8 indicates that the divergent effects of manuring under varying light levels become progressively greater with time, and for this reason the data for the last sampling occasion (Table VIII) reflect the interaction more markedly than the mean data for all samples (Table VII).

Apart from the interactions between the light and nutrient supply factors on plant growth, there are also significant interactions between nitrogen and

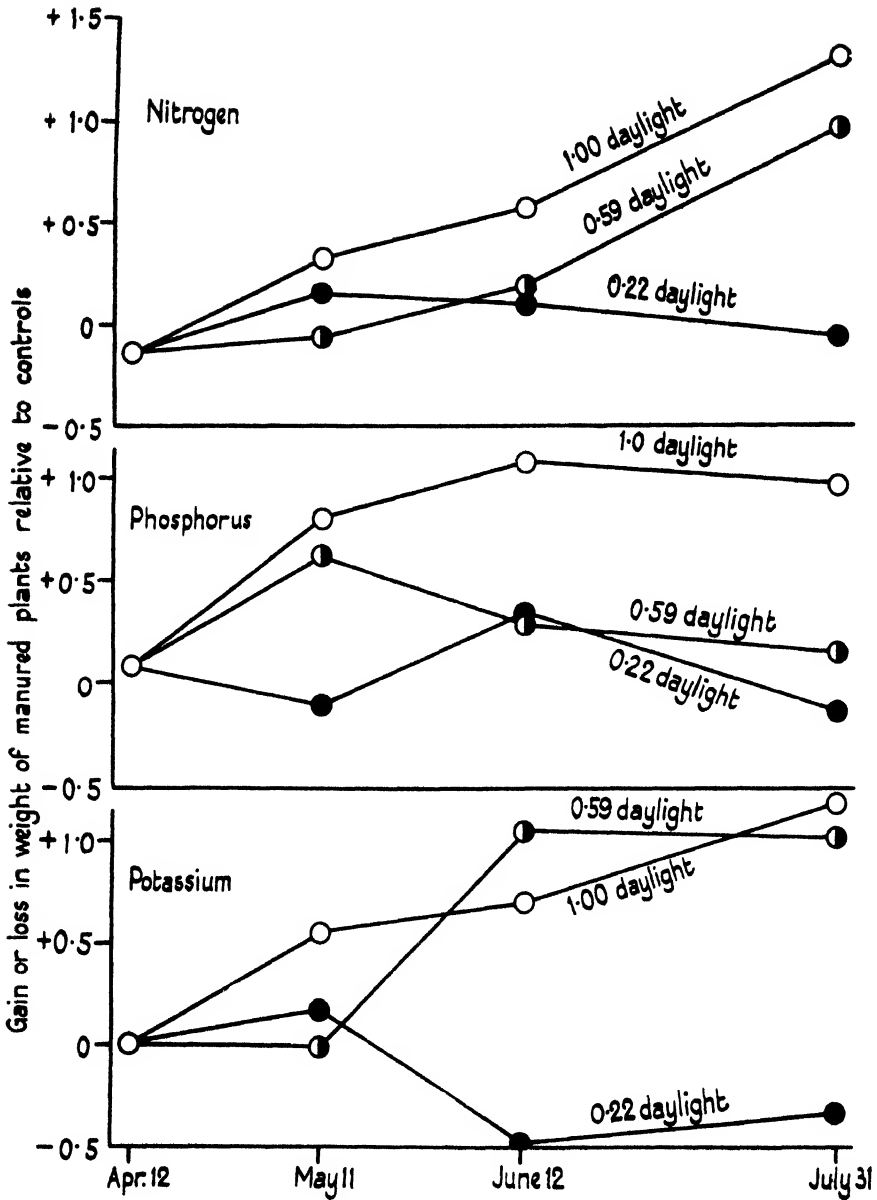


FIG. 8. The interaction between mineral nutrient supply and light intensity in the seasonal growth of *S. non-scripta*. The effects of additional nitrogen, phosphorus, and potassium are expressed as the gains or losses in weight of manured plants over control plants at each of the three levels of light intensity (expt. IV, 1939).

TABLE VIII

The Interaction between Light Intensity and Mineral Nutrient Supply in the Final Weight of the Bulb

Experiment IV, 1939

Light intensity.		Dry weight of bulb (gm.), sample IV.					
		Nitrogen effect.		Phosphorus effect.		Potassium effect.	
		N.	No N.	P.	No P.	K.	No K.
1.00 daylight	Bulb weight	6.96	5.66	6.80	5.82	6.91	5.72
	Bulb weight, log. scale	0.835	0.751	0.824	0.762	0.833	0.753
0.59 daylight	Bulb weight	6.73	5.82	6.39	6.16	6.81	5.74
	Bulb weight, log. scale	0.824	0.754	0.790	0.788	0.831	0.747
0.22 daylight	Bulb weight	4.00	4.00	3.91	4.09	3.86	4.14
	Bulb weight, log. scale	0.600	0.596	0.587	0.609	0.583	0.613

Log. scale: significant difference between light intensity \times nutrient supply means = 0.069

phosphorus, and phosphorus and potassium. The relevant data are set out in Table IX; it is seen that phosphorus has a greater effect in the presence of nitrogen and similarly potassium has a greater effect in the presence of phosphorus.

TABLE IX

The Interactions of Nitrogen with Phosphorus and of Phosphorus with Potassium in the Growth of Flowering Plants

Experiment IV, 1939

Mean plant weight (gm.) over all light intensities and sampling occasions.

Untransformed data.	N.	No N.		P.	No P.
P.	5.72	5.03	K.	5.72	4.95
No P.	4.97	4.92	No K.	5.02	4.94
Log. data.	N.	No N.		P.	No P.
P.	0.733	0.676	K.	0.730	0.677
No P.	0.676	0.674	No K.	0.679	0.674

Log. scale: significant difference between means ($P = 0.05$) = 0.035

The interrelated effects of light intensity and mineral nutrient supply on total plant growth are reflected in the data for leaf weight. As in 1938 (expt. III—Fig. 5), the interaction between light intensity and occasion on leaf weight is marked. In Fig. 9 it is seen that at the time of flowering (May 11) shading had no effect, but a month later (June 12) at the higher light intensities the leaf weight decreased significantly—due to the earlier onset of senescence.

In addition to the light effects, nutrient supply also caused significant changes in leaf production. The influence of additional nitrogen, phosphorus,

and potassium on the mean leaf weight of plants at different light levels are shown in Fig. 10 and Table X, where the logarithmic transformation has been employed for the statistical calculations of errors. The over-all effect of both nitrogen and potassium is to increase leaf weight, but in the case of phosphorus the result is not significant. There is some indication of an interaction between nutrient supply and light intensity only in the case of potassium and phosphorus, but these interactions are not significant.

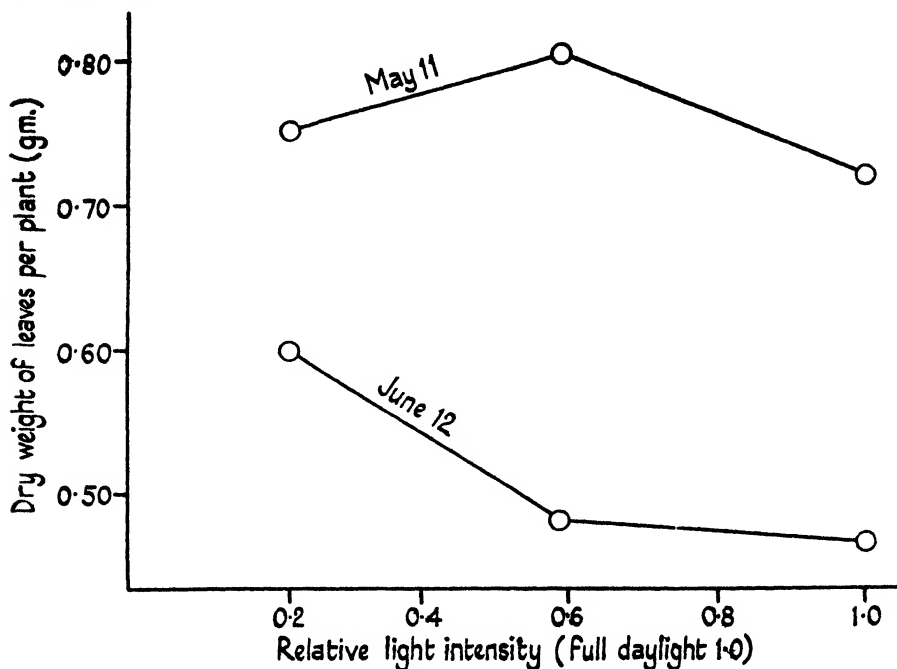


FIG. 9. The effects of varying light intensity on leaf weight of *S. non-scripta* at flowering (May 11) and a month later (June 12) (expt. IV, 1939).

TABLE X

The Effects of Light Intensity and Mineral Nutrient Supply on Leaf Production

Experiment IV, 1939

Manurial treatments.		Log. of mean leaf dry weight (gm.) of 5 plants.			
		Light intensity (daylight = 1.0).			
		1.00.	0.59.	0.22.	Mean.
Nitrogen	.	0.503	0.511	0.563	0.526
No nitrogen	.	0.394	0.449	0.461	0.434
Phosphorus	.	0.483	0.483	0.515	0.494
No phosphorus	.	0.414	0.477	0.509	0.466
Potassium	.	0.488	0.516	0.521	0.509
No potassium	.	0.408	0.443	0.503	0.452
Significant difference			0.089		0.051
(P = 0.05)					

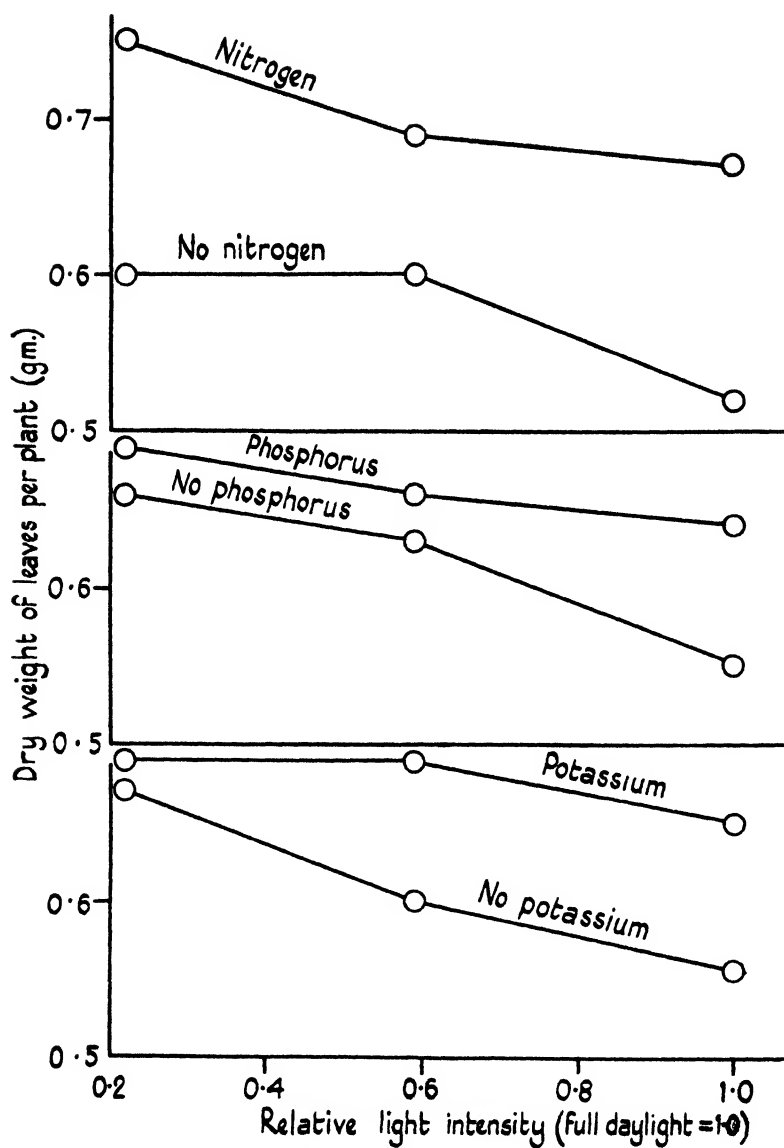


FIG. 10. The effects of additional nitrogen, phosphorus, and potassium at varying levels of light intensity on the leaf weight of *S. non-scripta* (expt. IV, 1939).

Finally in 1939 there was a significant interaction between the levels of phosphorus and potassium. At only a high level of potassium does phosphorus increase leaf production—see Table XI.

TABLE XI

*The Interaction of Phosphorus and Potassium in Leaf Production**Experiment IV, 1939*

Mean leaf dry weight (gm.) per 5 plants: untransformed data.

	Phosphorus.	No phosphorus.
Potassium . . .	3.67	3.07
No potassium . . .	2.98	3.07

Log. data.

Potassium . . .	0.549	0.468
No potassium . . .	0.438	0.465

Log. data: significant difference ($P = 0.05$) = 0.073

Contrary to the 1938 results, the development of the inflorescence varies with the light level. From Table XII it is clear that the most-shaded plants have the lowest inflorescence weight but an intermediate rate of seed production.

TABLE XII

*The Effects of Varying Light Intensity on Inflorescence Weight and Seed Production**Experiment IV, 1939*

Light intensity.	Weight (gm.) of inflorescence.		Weight (gm.) of seeds.
	May 11.	June 12.	
1.0 daylight (L 1)	0.366	0.769	0.310
0.59 " (L 2)	0.490	0.614	0.051
0.22 " (L 3)	0.310	0.441	0.105
Significant levels.			
L 1-L 3	$P < 0.05$	$P < 0.01$	—
L 1-L 2	$P < 0.01$	—	—
Significant difference	($P = 0.05$)	—	0.048

In addition to the light factor, seed production was also affected by nutrient supply. Both phosphorus and potassium increased the seed weight per plant—see Table XIII. In the case of potassium, but not phosphorus, there was a significant interaction with light intensity. The individual seed weight was little affected by the experimental treatments, averaging 4.64 gm. per thousand.

The results of the corresponding 1940 experiment (expt. V) require somewhat different treatment for their interpretation since within each of the 48 plots there were 3 subplots containing bulbs taken from three different woodland sites. It is not, however, proposed to discuss the interrelationship between the effects of 'strain' and the light and manurial factors since these will be dealt with fully in a subsequent paper. The main effects are, however, relevant to the present discussion and will therefore be described briefly.

TABLE XIII

The Interaction between Light Intensity and Phosphorus and Potassium Supply in Seed Production

Experiment IV, 1939

Seed production (gm. per plant).

		Daylight.			Mean.
		1.00.	0.59.	0.22.	
Phosphorus	.	3.53	0.47	1.25	1.75
No phosphorus	.	2.67	0.55	0.85	1.36
Potassium	.	3.78	0.45	1.09	1.77
No potassium	.	2.43	0.58	1.02	1.34
Significant difference between fertilizer and no fertilizer means = 0.39					
"	"	"	potassium × light intensity means = 0.48		

Since at planting time the mean size of the bulb from the three woodland sites differed to some extent, in order to eliminate this variation the efficiency indices of Blackman (1919) have been calculated from the data of the first two sampling occasions—April 23 to May 28. The effects of shading in reducing the rate of growth of the whole plant is seen in Fig. 11 and it will be observed that between full daylight and 0.68 of daylight there is no change in the growth rate. Apart from the light effect, statistical analysis of the data revealed that there were no significant effects of additional nitrogen, phosphorus, or potassium.

For measuring the changes in growth over the whole season the ratio of the final weight at midsummer to the initial weight at planting was employed. In this instance, apart from the light effects, there were significant changes due to manuring. From Fig. 12 and Table XIV it is evident that phosphorus exerted a positive effect and that light intensity and nitrogen supply were interdependent. It should also be noted that a reduction in light intensity to 0.68 daylight has been beneficial.

TABLE XIV

The Interaction between Light Intensity and Nitrogen and Phosphorus Supply in Growth

Experiment V, 1940

Log. ratio of final to initial weight × 10.

		Daylight.			Mean.
		1.00.	0.68.	0.22.	
Nitrogen	.	1.32	1.34	1.22	1.29
No nitrogen	.	1.25	1.35	1.21	1.27
Phosphorus	.	1.30	1.35	1.24	1.30
No phosphorus	.	1.26	1.33	1.19	1.26
Mean	.	1.28	1.34	1.22	
Significant difference between light means = 0.04					
"	"	"	nutrient supply means = 0.03		
"	"	"	light × nitrogen-supply means = 0.06		

Independent of light intensity, nitrogen and phosphorus showed a significant interaction (Table XV), the effect of either being greater in the presence of the other.

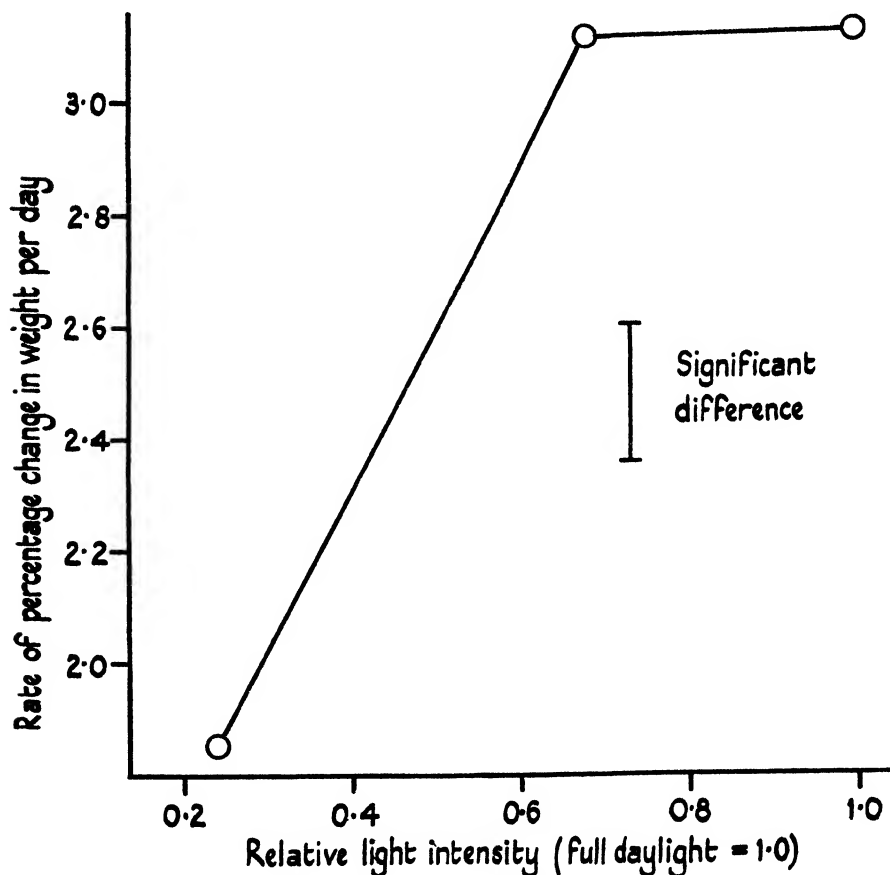


FIG. 11. The effects of varying light intensity on the rate of growth of *S. non-scripta* during April and May—growth-rates calculated as the efficiency indices (expt. V, 1940).

TABLE XV

The Interaction between Nitrogen and Phosphorus Supply in Seasonal Growth

Experiment V, 1940

Ratio of final to initial weight.

				Phosphorus.	No phosphorus.
Nitrogen	.	.	.	2.25	1.86
No nitrogen	.	.	.	1.93	1.92

Log. transformation.

Nitrogen	.	.	.	1.328	1.254
No nitrogen	.	.	.	1.271	1.268

Log. scale: significant difference = 0.047

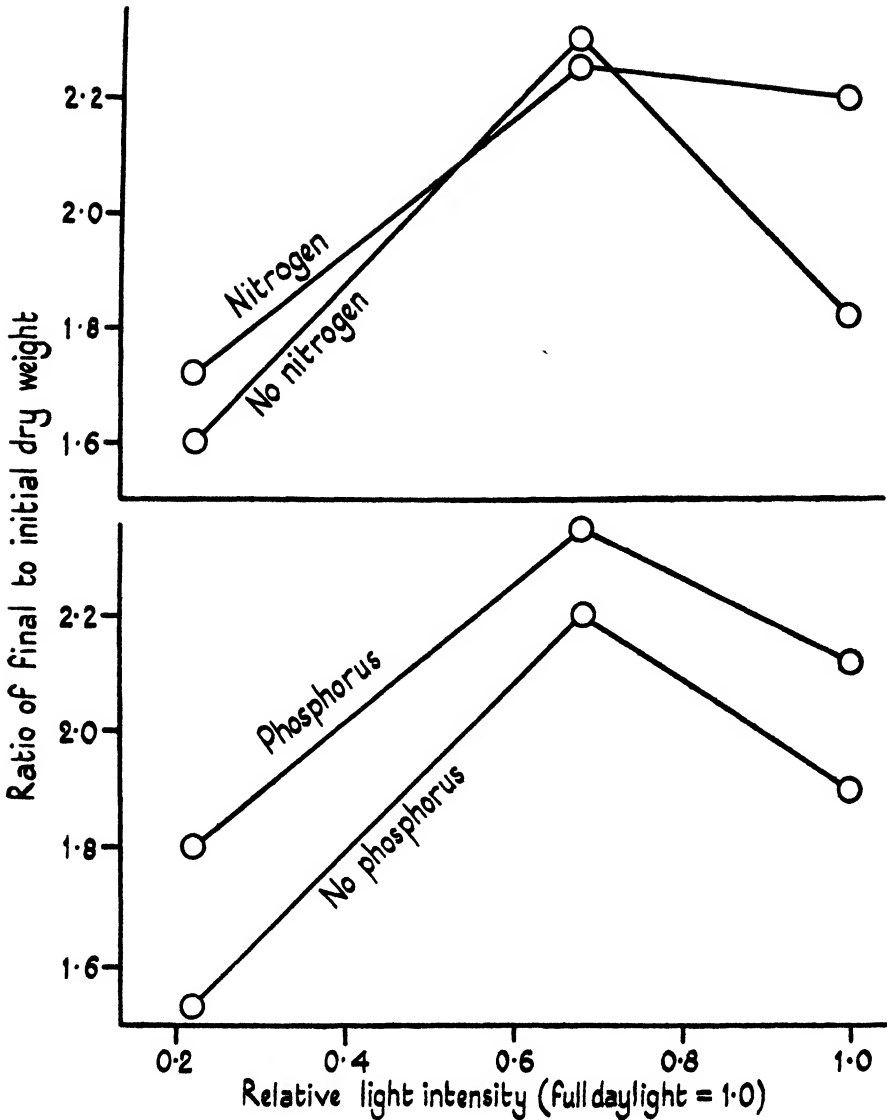


FIG. 12. The effects of additional nitrogen and phosphorus supply at different levels of light intensity on the seasonal growth of *S. non-scripta*. Changes in plant weight expressed as the ratio of final weight in midsummer to the initial weight in the previous autumn.

Considering the effects of light intensity and nutrient supply on leaf production, light intensity alone had no significant effect. There is, however, evidence that additional nitrogen increases leaf production more especially at the lower light levels. Moreover, there is a positive interaction between phosphorus and potassium (Table XVI).

TABLE XVI
The Effects of Mineral Nutrient Supply on Leaf Production

Experiment V, 1940

Log. dry weight (gm.) of leaves of 10 plants.					
Daylight.					
	1.00.	0.68.	0.22.	Mean.	
Nitrogen . . .	0.743	0.777	0.761	0.760	
No nitrogen . . .	0.738	0.726	0.728	0.731	

Significant difference ($P = 0.05$) between nitrogen and no nitrogen means = 0.051

Log. dry weight (gm.) of leaves of 10 plants.		
	Phosphorus.	No phosphorus.
Potassium . . .	0.549	0.468
No potassium . . .	0.438	0.465

Significant difference ($P = 0.05$) = 0.073

Finally, the only factor to affect flower production was light intensity. The flower weight fell progressively from 0.165 to 0.119 between full daylight and 0.22 daylight.

A further experiment was carried out in 1940 (expt. VI) which was concerned with the possible interaction between the effects of shading and strain on seasonal growth. Leaving aside the difference between bulbs collected from five woodland sites, the over-all effect of increasing shade on the gain in weight during the annual cycle is shown in Fig. 13. Gain, as in experiment IV, has been expressed as the ratio of the final bulb weight in midsummer to the initial bulb weight at the time of planting in the previous autumn. In addition, the data transformed to a logarithmic scale have been included in the figure together with the significant difference between treatments. It is clear that a reduction in the light intensity to 0.68 of daylight has caused no change but that further reductions in light intensity to 0.22 and 0.11 of daylight have progressively decreased the seasonal rate of increase.

3. *The effects of light intensity and mineral nutrient level on the seasonal growth of immature non-flowering plants*

On general grounds it seemed probable that small seedling bulbs, in which flower initiation was as yet absent, might react differently to the experimental conditions since the excess of assimilatory products would be solely concerned with bulb and leaf production and not with flower and seed formation. Accordingly in 1939 and 1940 parallel experiments were conducted with very small bulbs ranging in initial size up to 0.06 gm. Previous attempts had been made to use seed, but the germination in the spring of seed collected the previous summer was erratic.

In the 1939 experiment (expt. VII), besides the usual eight factorial combinations of nitrogen, phosphorus, and potassium, there were four levels of light intensity, making in all 32 treatments. Because of the small size of the

bulbs, sets of 5 bulbs were planted in land-drain sections (12 in. long and 4 in. diam.) sunk nearly flush with the soil surface. Each land drain, prior to use, was dipped in molten paraffin wax to ensure that there was no outward diffusion of salts. The treated drains were sunk in 24 groups of 8 and filled with soil. The 8 manurial treatments were applied at random to the individual pots in each group under the appropriate screen. Before the screens were placed on the pots (April 18) an initial sample of one plant was taken

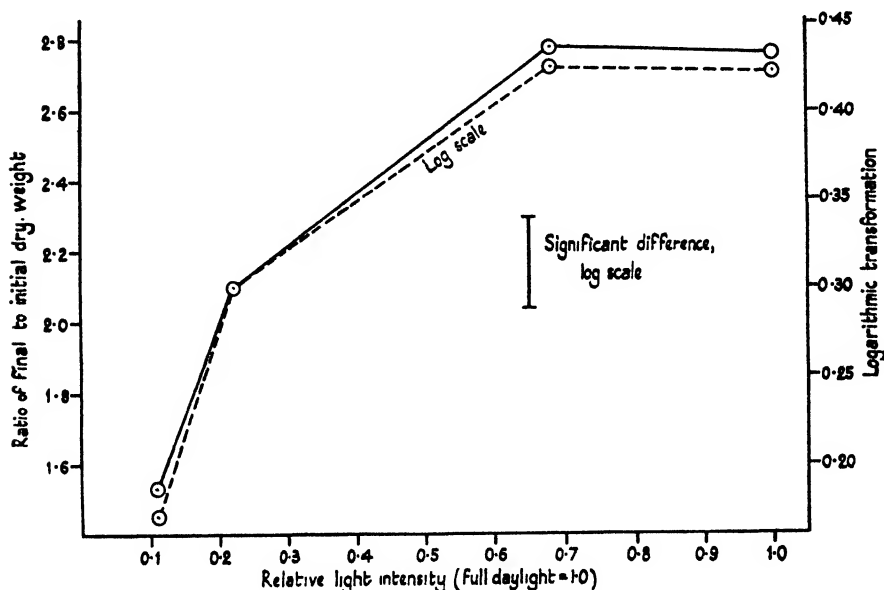


FIG. 13. The effects of varying light intensity on the seasonal growth of *S. non-scripta*. Changes in plant weight expressed as the ratio of final weight in midsummer to the initial weight in the previous autumn (expt. VI, 1940).

from each pot of 2 out of the 6 blocks. Subsequently the remaining plants in these two blocks were sampled on May 23 and the four other blocks when no leaves remained.

The effects of shading on the growth of these young plants are shown in Fig. 14 and Table XVII. At the time of the second sampling occasion there is a significant decrease in plant size with falling light intensity, but by the end of the growing season only a decrease in light intensity below 0.59 of daylight causes a significant reduction in plant weight.

It is especially noteworthy that at all light intensities the relative gain in weight over the whole season is higher than that recorded in the corresponding experiment with flowering bulbs (expt. IV). Compared with the initial weight at planting, the bulbs growing under 0.59 and full daylight have quadrupled in size by the following August. Moreover, at even 0.22 of daylight the weight is doubled and there is an increase of a third at the lowest light intensity of 0.11 daylight.

In spite of the marked effects of varying light intensity there were no significant responses to additional nutrient supply. Nor were there any significant interactions with light intensity.

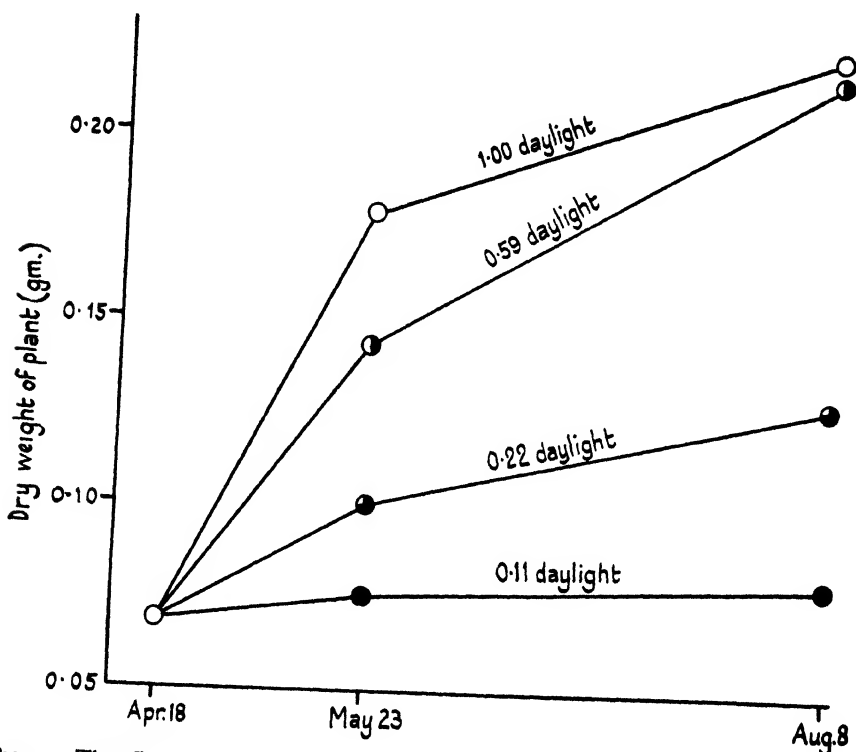


FIG. 14. The effects of varying light intensity on the seasonal changes in weight of immature non-flowering plants of *S. non-scripta* (expt. VII, 1940).

TABLE XVII

The Effects of Varying Light Intensity on the Seasonal Growth of Immature Non-flowering Plants

Experiment VII, 1939

Light intensity.	Log. mean dry weight (gm.) per 100 plants.	
	May 23.	August 8.
1.00 daylight	1.245	1.342
0.59 "	1.151	1.327
0.22 "	0.986	1.030
0.11 "	0.867	0.893
Significant difference	0.068	0.061
($P = 0.05$)		

As in all previous experiments, the changes in total plant weight were reflected in the bulb weight. In the case of leaf production there were no significant changes due to increased shading (Table XVIII). There was, however, some indication of an interaction between shading and potassium.

This interaction is, however, only significant at the $P = 0.05$ level, and the real significance of a negative effect of potassium with increasing light intensity is doubtful.

TABLE XVIII
*The Interaction between Light Intensity and Potassium Supply in Leaf
Production of Non-flowering Plants*

Experiment VII, 1939

		Dry weight (gm.) of leaf per plant.			
		Daylight = 1.0.			
		1.00.	0.59.	0.22.	0.11.
Potassium	.	0.0198	0.0152	0.0181	0.0181
No potassium	.	0.0220	0.0223	0.0220	0.0163
Mean	.	0.0209	0.0188	0.0201	0.0172
		Log. transformation (100 plants).			
Potassium	.	0.289	0.169	0.250	0.257
No potassium	.	0.324	0.343	0.327	0.185

Significant difference ($P = 0.05$) = 0.110

In 1940 a simpler experiment (expt. VIII) was carried out in which there were three blocks and three light intensities, viz. 1.00, 0.68, and 0.22 of daylight, making 9 groups of pots. Each group contained 8 pots, 4 of which received no additional mineral nutrients while the remainder all received nitrogen, phosphorus, and potassium at the standard rates. The pots were not waxed, and in spite of watering the plants showed signs of water deficiency. This droughting effect was most noticeable in the unshaded pots and the leaves died back a month earlier than those of the shaded plants. In consequence of the longer assimilating period at the reduced light intensities no significant effect of light level was recorded; nor was there any significant change due to nutrient supply (see Table XIX).

TABLE XIX
*The Effects of Varying Light Intensity and Nutrient Supply on the Seasonal
Growth of Immature Non-flowering Plants*

Experiment VIII, 1940

		Final dry weight (gm.) of plant.			
		Daylight = 1.0.			
		1.00.	0.68.	0.22.	Mean.
N.P.K.	.	0.364	0.390	0.373	0.375
Control	.	0.312	0.480	0.347	0.380
Mean	.	0.338	0.435	0.360	

No significant differences

DISCUSSION

Although the bluebell is a characteristic feature of the ground flora of much English woodland it is clear from the present investigations that it can in no way be classed among the obligate shade plants of Lundegårdh (1931).

Rather the experimental results indicate that *S. non-scripta* behaves like a typical 'sun' plant. In fact, over the period of most active growth during April and May it is seemingly more susceptible to shade than many of the species examined by previous workers.

In 1938 a reduction in the light intensity to 0.47 of daylight caused a significant decrease in the rate of growth (Fig. 3 and Table III). In the 1939 experiments, both with flowering plants and with small plants which had not yet reached the flowering stage, growth during the season was as great in 0.59 as in full daylight (Figs. 7 and 14, Tables V and XVII). In Fig. 8 and Table VIII there is, however, some indication that where mineral nutrient supply was not limiting growth the plants were smaller at 0.59 of daylight.

In two of the 1940 experiments (expts. V and VI) a fall in light intensity from full daylight to 0.68 of daylight caused no decrease in size, but a further increase in shading to 0.22 of daylight led to a marked diminution of growth (Figs. 11 and 13). Because of the large interval between 0.68 and 0.22 of daylight it is not possible from Figs. 11 and 13 to judge the critical light level below which there would be a reduction in growth. By fitting, however, equations of the form $Y = a + bx + cx^2$ to the data, it has been estimated that below an intensity of half-daylight growth is decreased. There is thus general agreement in the three seasons that bluebells will be retarded during the most active growth phase by a light intensity of half-daylight.

A comparison of these present results with the findings of previous workers is hampered in several ways. In few instances have the past measurements of growth been so precise, and in most cases it is not possible to assess the experimental errors. Furthermore, the experiments were not planned on multifactorial lines, and there is rarely any means of judging whether other factors than light intensity were limiting growth.

The most relevant field studies are Muckerji's (1936) investigations on the autecology of another woodland plant, *Mercurialis perennis*. Muckerji deduced from his data that the maximum shoot weight for female plants occurred in woodland sites where the degree of shade during the 'high light' phase was 0.2–0.4 of daylight, whereas for male plants a higher light intensity was optimal—0.5–0.7 of daylight. As, however, the 'high light' phase was defined as the period February to May and only a single light intensity determination—on unspecified dates within this period—was made in the widely scattered localities, the error of the light estimate must have been exceedingly high.

Again a single determination in the early spring is difficult to correlate with the average intensity during the whole period of growth, which in the case of *M. perennis* extends from the spring to the autumn. According to the present investigations the fall in light intensity with the expansion of the canopy is very considerable. In the open oak wood (expt. I) the reduction in light intensity between March and June was 62 per cent. and comparable reductions of 80–96 per cent. were recorded for three closed woods in the previous paper. Taking this seasonal fall into account it would seem that the female

plants of *M. perennis* in particular are more tolerant of shade than the bluebell.

On the other hand, with a varying light level, changes in shoot weight do not proportionately reflect changes in plant weight. Muckerji points out that the shoot growth of *Mercurialis* in the spring is dependent in part on the translocation of reserves from the roots and rhizomes. Results, as yet unpublished, on other plants indicate that light and temperature are important factors in determining a widely varying ratio of shoot to root.

Finally, if Muckerji's technique had been applied to bluebells in woodland—admittedly a very different type of plant to *Mercurialis*—the result would have established that shoot weight was not related to light intensity (see Figs. 5, 6, and 9 and Table V). It might, therefore, have been concluded erroneously that the bluebell is tolerant of a very wide range of light intensity.

A number of investigations have been concerned with the growth of plants where the light intensity has been reduced by shading either in the greenhouse or in the open. Clements and Long (1934) demonstrated that a reduction in the light intensity from daylight to 0.32, 0.16, and 0.08 of daylight progressively decreased the weight of sunflower (*Helianthus annuus*) plants, and that this fall was less marked when water-supply was limiting growth. Because, however, of the distribution of the light values it cannot be concluded at what point the light intensity initiated a fall in plant weight. Shirley (1929) grew seedlings of a large number of plants in unreplicated experiments under screens, both in the open and in the greenhouse. In the greenhouse, where the range of intensities was from unscreened plants (0.71 of daylight) to 0.01 of daylight, he found that the weight produced was almost proportional to the light received under the conditions of early spring. In summer *Geum canadense* and *Lycopersicum esculentum* showed less response to a change in light intensity above 0.4 of daylight than below the level. But with a number of other plants, e.g. *Sequoia sempervirens*, *Helianthus cucumerifolium*, *Pinus Taeda*, *Galinsoga parviflora*, there was a linear relationship between dry weight and light intensity. Porter (1937), also working under greenhouse conditions, found that with tomatoes there was only a small loss in weight per plant when the light intensity was reduced to 0.78 per cent. of the full intensity inside the house.

In investigations with plants grown out of doors Shirley (1929) reported that there was little increase in plant weight above a value of 0.47 daylight. For some species such as *S. sempervirens* and *G. canadense* maximum growth was made at 0.47 and 0.74 of daylight respectively. Blackman and Templeman (1938) found that when *Agrostis tenuis* and *Trifolium repens* were defoliated at frequent intervals, decreasing the light level from daylight to 0.61–0.63 of daylight caused large and statistically significant depressions in leaf regeneration. Benedict (1941) grew three range grasses—*Agropyron cristatus*, *A. Smithii*, and *Bouteloua gracilis*—under a series of shades. With *B. gracilis* the weight increase was linear between half and full daylight, but

with the two *Agropyron* species the difference between 0.7 and full daylight was not marked.

The only evidence that conflicts with the general trend of the investigations so far cited is the claim made by Panchaud (1934) that with radishes (*Raphanus sativus*) the gain in weight between 0.75 and daylight was much greater than the gain in weight between the interval 0.5 and 0.75 of daylight. The experiment was, however, unreplicated and from inspections of the data the errors would appear to be high. This exception does not, however, invalidate the general evidence that the bluebell in its reaction to light intensity is as sensitive as, if not more sensitive than, plants normally found growing in open situations.

Considering the light factor over the whole of the growth cycle of the bluebell, the earlier senescence of the leaves observed in the unshaded plants must be taken into account. The extension of the assimilatory period at the level of 0.59–0.68 of daylight has more than offset the shading effect in several experiments since the final bulb weights do not differ significantly from those of plants receiving full daylight. There is considerable indirect evidence that this earlier dying back of the leaves in full daylight is associated with a limitation of water-supply. In 1938—a wet year—the interval between the initiation of senescence of unshaded and shaded plants was shorter than in the dry season of 1940, and this seasonal contrast is reflected in the relative effects of shading on growth. In 1938 (expt. III) the final weight of the bulb was depressed by a reduction in light intensity to 0.47 of daylight (Table III), whereas in 1940 (expt. V) the bulb weight has been increased significantly when the plants were shaded (0.68 of daylight)—see Table XIV.

That shading results in a considerable decrease in the transpiration rate has been demonstrated by a number of investigators, e.g. by Clements and Long (1934) and Martin (1935), working with the sunflower, and by Biswell (1935) investigating seedlings of eight deciduous trees. How far these results were dependent on the methods of screening used and the size of the plots cannot be judged. There is little doubt that any technique of screening will tend to lower the air temperature when the air is still, even if the screens are small. There will, too, be a diminished loss of soil moisture since the soil temperature will also be less.

Under woodland conditions, this advantage of shade might be considered to compensate to some extent for the lower light level, especially in periods of deficient water supply. However, for the first half of the spring bluebells, except for freshly germinated seedlings on the soil surface, should not lack available water on account of the soil reserves from the winter rains. It is later in the season that soil moisture may become a more important factor. On the other hand, as the availability of water falls so does the light intensity. It was shown in the previous paper (Blackman and Rutter, 1946) that in the three deciduous woods, where the canopy was *closed*, even in the initial high light phase the light intensity ranged from 0.44 to 0.25 of daylight; a level of light intensity which the present experiments have shown limits the

growth of the bluebell even in a dry spring. Moreover, as the degree of shade was increased from 8 to 20 times by the time the canopy was fully expanded, there is no doubt that with the expansion of the canopy light must have become an even more dominant factor in limiting growth.

In woodland with an open canopy, such as in experiment I, and in the open chestnut coppice described in the previous paper, the light intensity did not fall to a figure of half-daylight until half-way through the season. It can, therefore, be concluded that the light factor in such types of woodland does not become operative until the plants are in flower.

Apart from the question of the light intensity, the present experiments provide information on the relative importance of nutrient supply. In none of the experiments did additional nitrogen, phosphorus, or potassium produce large effects on growth. There was in 1938 a very small response to nitrogen in experiment III (Table IV). Larger responses to nitrogen, phosphorus, and potassium were recorded in 1939 for the flowering plants (expt. IV), but none were found with the immature plants (expt. VII). Again, in 1940 there was in experiment V a small increase in final bulb weight over all light intensities due to additional phosphorus.

The failure of the bluebells to respond markedly to additional nutrients cannot be ascribed to the high fertility of the Thames Gravel soil. Prior to the experiments, the land had been neglected, ungrazed grassland which had received no fertilizers for many years. Moreover, in the latter half of 1940 the same series of plots were sown with sunflowers which, as other investigations in progress have demonstrated, do not demand a high level of soil fertility. Nevertheless the yield data in Table XX demonstrate that the increases due to potassium, phosphorus, and nitrogen were enormous compared with the maxima of 5–20 per cent. obtained with bluebells. Furthermore, during 1941–3, similar very large manurial responses were obtained with linseed (*Linum usitatissimum*), soya bean (*Soja max*), and maize (*Zea Mais*) on the same series of plots.

TABLE XX

*The Effects of Mineral Nutrient Supply on the Growth of
Helianthus annuus*

Fresh weight (gm.) at flowering.							
Control	.	.	42.9	NP	.	.	153.7
N	.	.	110.0	NK	.	.	125.6
P	.	.	175.9	PK	.	.	304.6
K	.	.	151.5	NPK	.	.	357.3

Translating the results of the 1938–40 experiments to woodland conditions, it appears most improbable that mineral nutrient supply can play more than a minor role. In the first place, it has been demonstrated that the effect of nutrient supply is often dependent on the light intensity. Experiment IV shows that whereas in full daylight there is a gain in the final bulb size due to additional nitrogen, phosphorus, and potassium there are no increases at

0.22 daylight (Table VIII). Similarly the increases in seed production brought about by nitrogen and potassium occur only in full daylight (Table XIII). In experiment III the small nitrogen effect on bulb size is independent of light intensity (Table IV), but in experiment V the nitrogen gain is greatest in full daylight, though this is not so for phosphorus (Tables VII and VIII). It can be concluded, therefore, that in closed woodland the low level of light intensity will tend to mask the factors of nutrient supply.

Even, however, in open woodland there is little ground for assuming the importance of mineral nutrients. In experiment I the bluebells failed to make any increased growth when nutrients were added. Moreover, in deciduous woodland there will be an annual return of such nutrients to the soil surface after leaf fall in the autumn. On the basis of the analyses of the leaves of 23 American deciduous trees shortly before leaf fall, McHargue and Roy (1933) calculated that 79 lb. of nitrogen, 47 lb. of potassium, and 11 lb. of phosphorus per acre would be returned on an assessed mean leaf fall of 2 tons per acre. Analyses by Coile (1937) of fallen leaves indicate that these estimates should be reduced by a third. Nevertheless, only in the case of phosphorus is the rate of return substantially below the rates of application made in the present experiments.

The release of the nutrients—more especially nitrogen—from the leaf litter will be dependent on the rate of decomposition and this in turn, as many workers have established, will be related to the pH value. In open woodland, if the soil is acid, nitrogen may therefore tend to limit the growth of seedlings and the very immature bulbs found in the surface litter.

There is, however, another aspect of the present investigations which points to a low nutritional requirement of the bluebell, namely, the extremely slow rate of growth. The ratio of final bulb weight in summer to the initial bulb weight, when planted the previous autumn, can be taken as a measure of annual growth, and the relevant data is summarized in Table XXI.

TABLE XXI

The Effects of Light Intensity on the Annual Growth of Bluebells

Ratio of final to initial bulb weight.

Light intensity (daylight = 1.0).

	1.0.	0.47-0.68.	0.20-0.22.	0.11.
<i>Flowering plants</i>				
Experiment III, 1938	1.98	1.61	1.10	—
„ IV, 1939	1.88	1.87	1.19	—
„ V, 1940	2.01	2.27	1.67	—
„ VI, 1940	2.76	2.78	2.10	1.53
<i>Non-flowering plants</i>				
Experiment VII, 1939	3.70	3.60	2.11	1.33
„ VIII, 1940	1.99	2.56	2.12	—

Taking first the results for mature flowering plants, it is seen that even in full daylight the weight is only doubled and that at 0.2 of daylight the average gain is only 52 per cent. With non-flowering plants the annual rate

of growth is, over all light intensities, slightly higher, but nevertheless the growth rates must still be considered as very slow compared with other liliaceous plants. For example, Holdsworth (1945) has shown that onion 'sets' will make a 10-40-fold increase in 20 weeks, while from Scott's (1943) account of *Allium vineale* it is clear that seasonal growth is also very rapid. Finally, other experiments in progress have shown that sunflower seedlings under English conditions are capable of doubling their weight in four days.

It is also of interest to note that in the woodland experiment (expt. I), where the light ranged from an initial figure of 0.84 of daylight to a final figure of 0.32 (mean value = 0.6), the seasonal gain in weight was 60 per cent.—a figure of very considerable interest since it is comparable with the results obtained in the field experiments.

Finally, it can be concluded that the results of the field experiments both confirm and amplify the results obtained in the woodland studies. The importance of the light factor in controlling the distribution of bluebells in *closed* woodland, as assessed by statistical field studies, is fully confirmed by the experimental determination of the susceptibility of *S. non-scripta* to shading. In fact, these parallel investigations serve to illustrate the benefit in ecological research of combining statistical observations in the field with controlled experiments involving essentially the examination simultaneously of several factors in laboratory, pot, and plot investigations.

SUMMARY

By the use of special statistical methods for the collection and interpretation of field data, it was demonstrated in the previous paper (Blackman and Rutter, 1946) that in three woodland sites with a *closed* canopy the distribution of the bluebell was largely determined by variations in the degree of shading. The present parallel investigations have been directed to assessing the effects of varying the light intensity and mineral nutrient supply on growth and development of the bluebell under more controlled conditions.

A preliminary study was first made in 1937 in an oak wood to determine the general trend of growth during the season. It was demonstrated that after a period of dormancy in the late summer new root growth was initiated by the bulb in the autumn and the elongation of the shoot commenced. By the time the shoot appeared above ground in March there had been a progressive loss in weight. It was not until mid-April that the plants started to gain weight, and this continued until the end of May. Subsequently, with the senescence of the leaves and the shedding of the seed, there was again a decrease in weight until the bulb entered the dormant phase. Additions of nitrogen, phosphorus, and potassium did not affect the rate of growth in the experimental sites where the light intensity fell from 0.84 of daylight in March to 0.32 of daylight in June. Under these conditions the average gain in plant size between March and the beginning of June was 200 per cent. and between March and July, after the seeds had been shed, 60 per cent.

Subsequent to these woodland investigations, some six multifactorial field experiments were carried out during 1938–40. Graded bulbs of flowering and non-flowering size, planted in open ground in the previous autumn, were subjected in the following spring to three or four levels of light intensity ranging from daylight to 0.11 of daylight. Coupled with the light treatment were eight balanced combinations of nitrogen, phosphorus, and potassium, viz. C, N, P, K, NP, NK, PK, NPK. Thus in the largest experiment there were 32 treatments. Moreover, for the determination of the changes in leaf, flower, and bulb weight the plots were subsampled up to five times during the season. The largest experiment involved planting some 2,400 bulbs, and the experimental treatments, including the occasion effects, amounted to 96.

During the most active phase of growth—April to mid-May—lowering the light intensity to 0.47–0.58 of daylight significantly decreased the weight of the bluebell in two out of three experiments. A reduction from full daylight to 0.68 daylight caused no loss in weight in the remaining three experiments. In five out of the six experiments a further decrease in the light intensity to 0.2–0.22 of daylight led to a sharp depression in the growth-rate.

Over the annual cycle bluebell bulbs increase in size very slowly. With mature flowering bulbs, between autumn and the next midsummer the weight is approximately doubled in full daylight, whereas at 0.2 daylight the mean annual gain is 52 per cent. Immature non-flowering bulbs have a higher rate of increase over all light intensities. In full daylight gains up to 270 per cent. have been recorded, with corresponding maximum increases of 160, 111, and 33 per cent. at 0.58, 0.22, and 0.11 of daylight.

In no experiment did the light factor affect leaf production; shading did, however, delay the onset of leaf senescence, especially in a dry year. Reducing the light intensity also tended to depress seed production.

There is good evidence that bluebells do not demand a high level of mineral nutrient supply. In only one out of the five experiments conducted on a gravel soil, known to be infertile, were the responses to additional nutrients appreciable; maximum increases in plant weight of 20–5 per cent. over the whole season were recorded for additional nitrogen, phosphorus, and potassium. In another experiment there was a small but significant nitrogen effect (4 per cent. increase). In two of these trials there was a significant and positive interaction between the phosphorus and nitrogen effects on final bulb weight and in one of the experiments a further positive interaction between phosphorus and potassium.

Apart from plant weight, leaf weight was increased to a small extent by additional nitrogen in two experiments and by phosphorus and potassium in one experiment where in addition there was a significant interaction between phosphorus and potassium. In one experiment seed production was significantly increased by additional phosphorus and potassium.

There is a marked interdependence between the nutrient effects and the degree of shading. In the experiment where there were appreciable gains in plant weight due to increased nutrient supplies, these were significant in

full daylight but *not* significant at 0.22 of daylight. Similarly, in another experiment nitrogen only increased the leaf weight in full daylight, and in a third trial the effect of potassium on seed production was confined to the unshaded plants.

It is concluded that the bluebell in its reaction to shading must be classed as a typical 'sun' plant and can in no way be regarded as an 'obligate shade' plant.

From the field investigations it directly follows that as growth is depressed at light intensities below half-daylight, in *closed* deciduous woodland light must be a controlling factor since the mean degree of shade is well below this critical value of half-daylight. Only in *open* woodland during the initial high light phase will growth not be limited by the light intensity. It is also improbable that in woodland the level of available mineral nutrient supplies will retard growth. Moreover, the effects of mineral nutrition will normally be masked by the light factor. The present experiments therefore confirm and amplify the conclusion reached in the previous woodland investigations that the distribution of the bluebell is largely determined by the degree of shading.

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Fritschiella tuberosa Iyeng.

BY

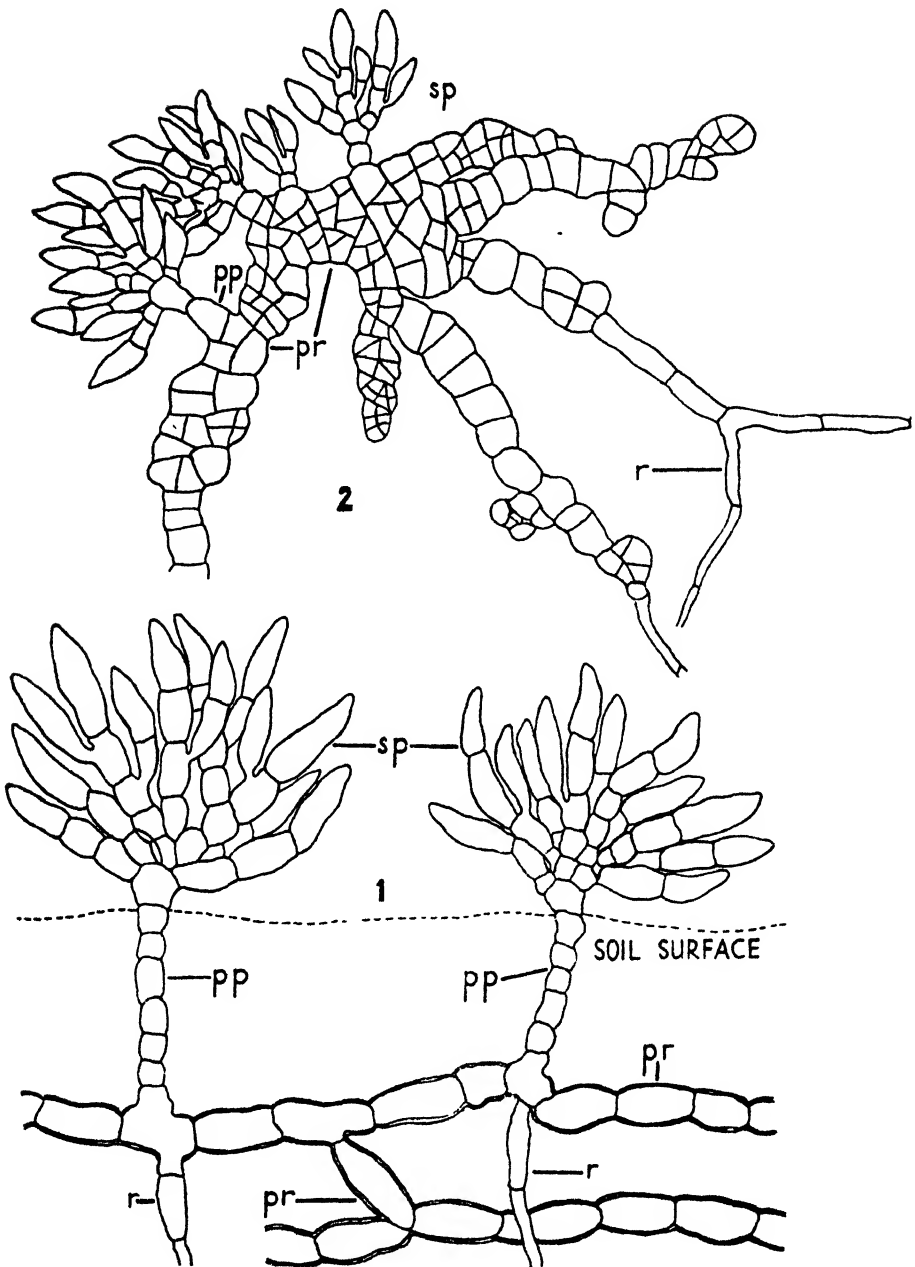
RAMA NAGINA SINGH

(Benares Hindu University)

With eleven Figures in the Text

THE monotypic terrestrial alga, *Fritschiella tuberosa*, first described by Iyengar in 1932 from the beds of drying rain-water pools on moist silt at Madras and Talguppa (Mysore Province), has since been collected by the present author from numerous and varied habitats in the United Provinces (cf. also Singh, 1941; Randhawa, 1939). A study of the alga from these sources has already revealed diverse important features in its morphology, structure (Iyengar, 1932; Singh, 1941), and reproduction (Singh, 1941). Further data regarding its cytology, life-cycle, development, perennation, and role in colonization have recently been obtained and form the subject of this paper. In a recent publication, Fritsch (1945) regards *Fritschiella* as of great phylogenetic importance in tracing the origin and evolution of higher land plants (cf. also Singh, 1941; Bower, 1935). On p. 6 of his paper he writes: 'In short *Fritschiella*, albeit in a somewhat specialized form, illustrates the existence of potentialities for a further elaboration in a Chaetophoraceous type in the direction above postulated for an early algal transmigrant (cf. also Bower, 1935, p. 498; Singh, 1941, p. 181).' The present study of *Fritschiella* lends further support to this view.

The alga consists of a plant-body differentiated into (1) a rhizoidal system, (2) a prostrate system, (3) a primary projecting system, and (4) a secondary projecting system. As originally described (Iyengar, 1932), the prostrate system is composed of a number of rounded or irregular swollen clusters of cells with dense contents and thin walls, the whole forming an irregular system with short congested branches. In a previous publication by the author (Singh, 1941), it was shown that it is this system which is concerned with reproduction and perennation. A reinvestigation of the prostrate system has thrown further light on its peculiar structure and function. It occurs in the form of uniseriate or multiseriate filaments, sometimes clearly differentiated into nodal and internodal parts (Fig. 1), or as irregular parenchymatous clusters (Fig. 2). In the former case, while the primary projecting system arises from the upper side at the nodes and ultimately ends in the fan-shaped subaerial green parts representing the secondary projecting system, the rhizoidal system arises from below the nodes (Fig. 1). The primary projecting system is transitory in character, possessing all the potentialities of structure and function of a prostrate one, into which it has at times actually

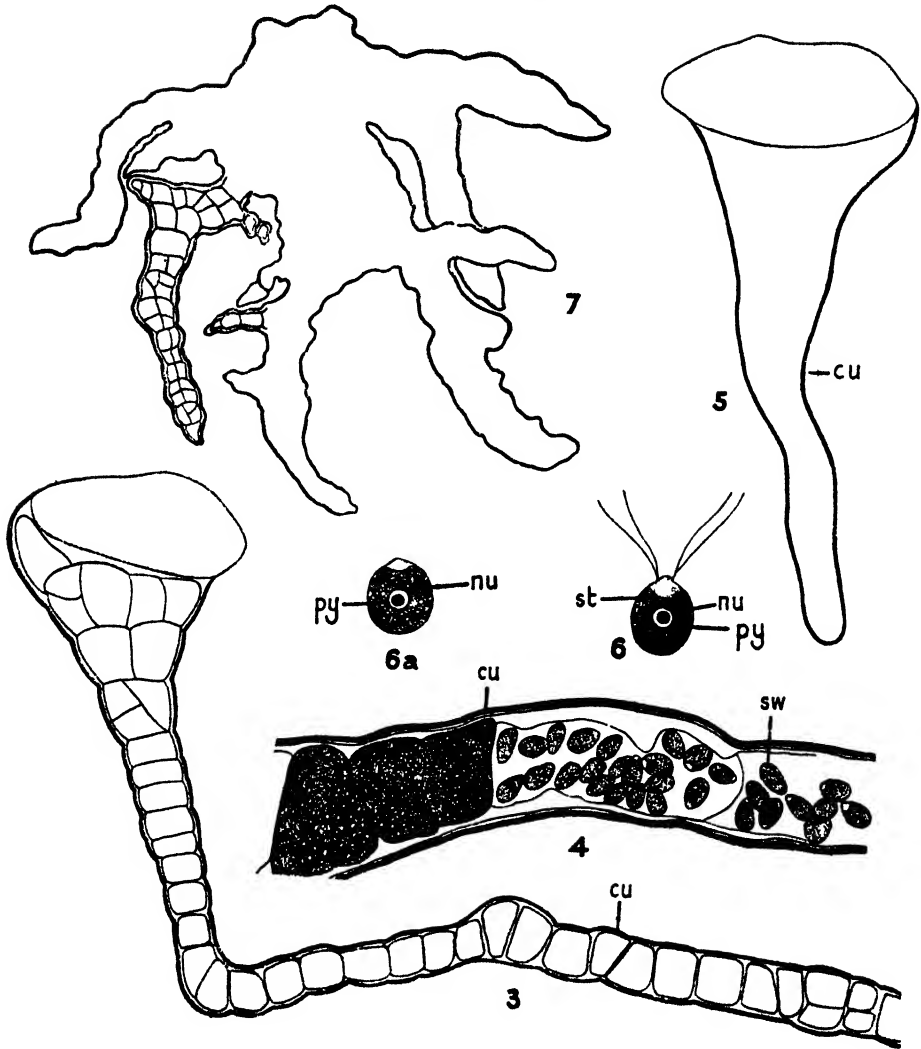


FIGS. 1 and 2. *Frittschiella tuberosa* Iyeng., mature plants. Fig. 1. Uniseriate prostrate system with clear differentiation into nodal and internodal parts and horizontal ramification. Fig. 2. Multiseriate irregularly parenchymatous prostrate system, partly vertical and partly horizontal in its ramification in the soil. *pp*, primary, and *sp*, secondary projecting systems; *pr*, prostrate system; *r*, rhizoids. (All approx. $\times 60$.)

been seen to be converted. The prostrate system ramifies extensively in the soil and forms a regular or irregular network, growing in the horizontal as well as the vertical directions. The capacity of the alga for colonization depends upon this feature which also makes the plant an efficient binder of the soil surface. In this respect, the alga may be compared either with the gametophytes of some Pteridophytes or the rhizomatous structures of some ferns and grasses.

The reproduction and perennation of the alga is brought about by the prostrate system, the cells of which are densely laden with food materials of a granular nature. Besides being capable of perennating as such, the prostrate filaments, in places, form multicellular club-shaped endings (Fig. 3) which, after the formation and subsequent liberation of swimmers (Fig. 4), usually appear as funnel-shaped structures (Fig. 5). These recall in appearance the cyst-forming vesicles of *Protosiphon* and *Botrydium* with which the alga is often associated and from which it cannot be easily distinguished, more especially in preserved material. Even in the fresh condition it is impossible to distinguish them unless the development of the structures in question is completely worked out. During the formation of these club-shaped structures there is secreted around them a thick pellicle of a dark-brown colour (Fig. 3), which possesses all the essentials of a true cuticle (Fritsch, 1944). The cells remain unaltered without change of shape or size (Fig. 4), although their contents become more homogeneous and brick-red in colour. With the onset of favourable conditions, especially access of moisture, the contents of the cells divide several times and the products eventually become converted into swimmers (Fig. 4). These are at first either colourless or slightly brick-red in colour, but after moving for some time they assume the usual green colour. They are quadriflagellate (Fig. 6), and in size, structure, and subsequent behaviour resemble to a great extent the quadriflagellate microzoospores formed during normal reproduction (cf. Singh, 1941, Fig. 32). In addition to the club-shaped perennating structures, tuber-like bodies (Fig. 8) are formed whose ultimate behaviour is similar to that of the former. Thus, *Fritschiella*, in possessing (1) a subterranean, brown, profusely branched, vertical or horizontal rhizomatous prostrate system, heavily laden with food materials and sometimes clearly differentiated into nodes and internodes, (2) a subaerial, green, fan-shaped projecting system, and (3) a downwardly directed rhizoidal system, the last two being restricted to the nodes, simulates the habit of some ferns and Gramineaceous plants.

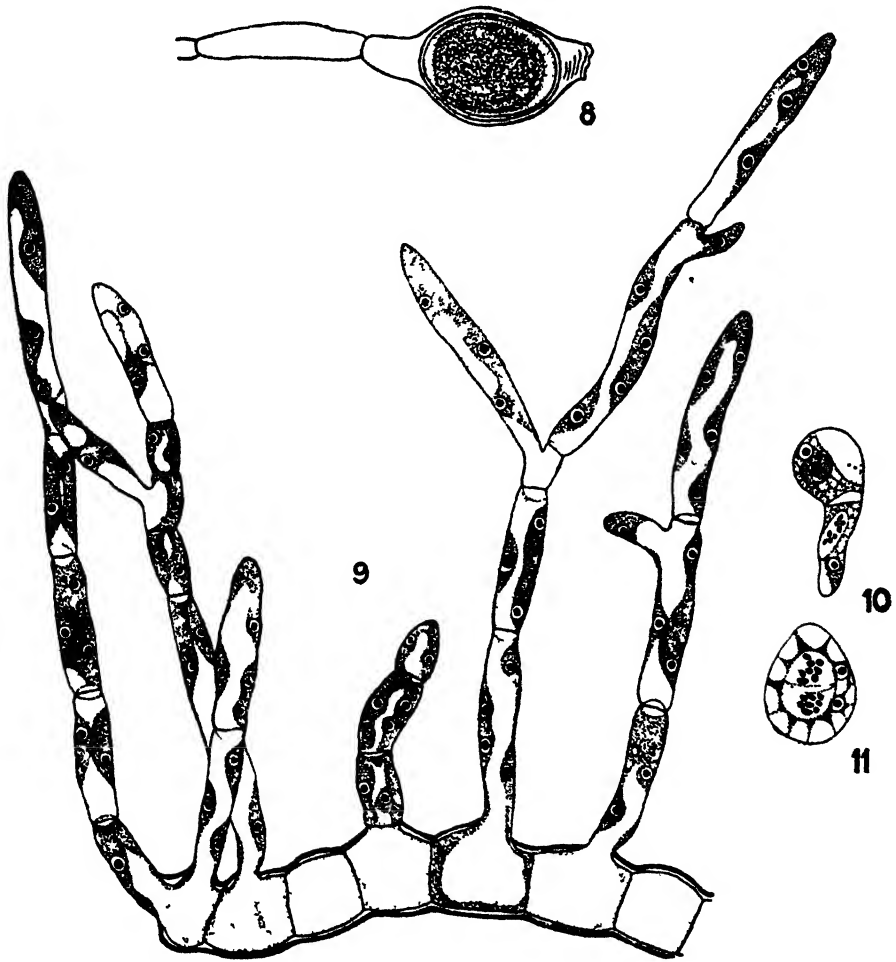
The perennating prostrate system sometimes produces plants directly without the formation of swimmers (Fig. 9). During such development a number of stages have been observed which have a striking resemblance to the genus *Oliveria* Nayal (1935), described from Egypt. The short-celled filaments of the latter alga, when exposed to drought, acquire thick walls and brown-coloured contents (cf. Nayal, 1935, Text-fig. 4), and then are exactly similar to the perennating prostrate system of *Fritschiella* (cf. Fig. 7). The similarity is further emphasized by the fact that reproduction (both asexual and sexual)



FIGS. 3-7. *F. tuberosa* Iyeng. Fig. 3. Portion of the perennating prostrate system with a club-shaped ending. Fig. 4. Part of the former showing swarmer-formation. Fig. 5. A funnel-shaped structure after the formation and liberation of swarmers. Fig. 6. Swarmer; 6a, swarmer with flagella withdrawn. Fig. 7. Perennating prostrate system which has undergone little change. *cu*, cuticle; *nu*, nucleus; *py*, pyrenoid; *sw*, swarmers; *st*, stigma. (Figs. 3, 5, and 7 approx. $\times 50$; 4 $\times 760$; 6 and 6a $\times 1,000$.)

is brought about only by the short-celled filaments (representing the prostrate system of *Fritschiella*) in *Oliveria*. In this genus Nayal (1935) has described the formation of quadri- and biflagellate zoospores and the fusion of biflagellate isogametes. Further, the occasional pigmented nature of the zoospores from cells with brown contents finds a parallel in the reddish swarmers produced from the perennating prostrate system of *Fritschiella*, described above.

Again, the two genera are similar in their general habit, in the methods of germination of zoospores and zygotes, in their germinals, and also in their frequent association, in nature, with *Protosiphon* and *Botrydium*. It thus



FIGS 8-11 *F. tuberosa* Iyeng. Fig 8 A tuber-like structure Fig 9 Direct germination of the perennating prostrate system Fig 10 Germination stage of a zoospore showing nuclear division with haploid chromosome complement Fig 11 Germination stage of zygote showing diploid chromosome complement (Figs 8 and 9 approx $\times 50$; 10 and 11 $\times 1,100$)

appears that *Frittschiella* and *Oliveria* may possibly be one and the same plant. While discussing the systematic position of *Oliveria*, Nayal writes: 'The cell groups and perennating cells in the short-celled filaments recall those of Iyengar's *Frittschiella*, but it again differs from *Frittschiella* in the absence of specialized structures like rhizoidal, prostrate, and projecting systems.' The absence of these specialized structures in *Oliveria* is perhaps a cultural effect,

since Nayal studied his plant only in artificial cultures. In view of the present writer's interpretation of *Oliveria*, considerable side-light is likely to be thrown on the geographical distribution and phylogenetic importance of *Fritschiella*.

In *Fritschiella* the zoospores and gametes are produced on different plants (Singh, 1941). The former germinate directly to give rise to new plants, while the latter fuse in pairs to form zygotes which, without undergoing any resting period, also germinate to form new plants. A cytological investigation of the germlings from the zoospores shows four chromosomes in their nuclei (Fig. 10), while the germlings from the zygotes possess eight chromosomes (Fig. 11). The reduction division thus appears to take place during zoospore formation in the plants produced by the germination of the zygotes. There is, therefore, a regular alternation of $2n$ and n generations, and since the two alternating phases appear outwardly identical, the alternation is isomorphic.

In conclusion, I have much pleasure in expressing my great indebtedness to Professor Y. Bharadwaja for his kind help and criticism during the preparation of this paper.

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The Effects of Illumination on the Respiration of Shoots of the Cherry Laurel

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With fifteen Figures in the Text

INTRODUCTION

THE effects of illumination and active photosynthesis on the respiration intensity in green leaves has long been a problem of great importance to the plant physiologist. Normal aerobic respiration, in its external manifestations, is the reverse of photosynthesis. Since therefore respiration continues in green tissues in the light, and provides some of the photosynthetic substrate (CO_2), a knowledge of the intensity of the respiration is essential to an accurate estimation of the real assimilation of the tissue in question. The importance of these facts has been fully realized by workers on assimilation, and numerous attempts have been made, by various indirect methods, to estimate the probable intensity of respiration in the light.

Two possible effects of illumination on the respiration of green tissues have been recognized. They are:

- (1) A direct effect on the respiratory system itself, stimulating or depressing the activity of one or more members of its enzyme complex, either directly or via the control of the protoplasmic organization. This type of effect has been called a plasmageneous effect.
- (2) An effect due to an increase of the respiratory substrate (sugars) produced in the assimilation of CO_2 . This has been called an ergastogeneuous effect.

Both these effects could result from illuminations either in the presence or the absence of carbon dioxide provided externally.

A considerable amount of research has been carried out on the plasmageneuous effect of illumination in a large variety of non-chlorophyllous plants. De Boer (1929), who has adequately reviewed all previous work, comes to the conclusion that the effect on such organisms as fungi, flowers, etiolated plants, &c., is nil. It is unlikely, however, that the reaction of these plants to light will be the same as that of green plants.

In investigations on the effect of illumination in green plants three main methods have been employed. They are:

- (1) Direct observations of the apparent intensity of respiration in the light. In these experiments the conditions, either natural or induced, of the

plant material were such that the respiration in the light was greater than the assimilation, resulting therefore in a net output of CO_2 . Thus Kniep (1914) found that diffuse sunlight greatly increased the respiration of various seaweeds (*Ulva lactuca*, *Fucus serratus*, &c.) which had been starved in the dark for 5 months. He regards this as a direct plasmageneous effect, made evident by the partial destruction of the assimilative capacity of the thallus. Much more recently van der Paauw (1932), working with the alga *Hormidium*, demonstrated that in diffuse light, either in the absence or the presence of external carbon dioxide, the oxygen intake of the alga was from two to three times that in the dark. From the magnitude of these effects, and from feeding experiments with hexose sugars, he concluded that the action of light was a plasmageneous one.

(2) Observations of the compensation point of assimilation and respiration. Thus Plaetzer (1918) found that there was a considerable increase in the light intensity of the compensation point as the temperature was increased, and that the relative increase of this light intensity was larger than the relative increase of the respiration in the dark over the same range of temperatures. He regarded this divergence as due to an increase in the respiration with increasing light intensity.

(3) Observations of the intensity of respiration of the tissues when placed in the dark after a period of illumination, deductions then being made as to the probable intensity during the illumination. Much the greatest amount of research has been by this method. Thus Borodin (1881) carried out extensive observations on *Crataegus* spp. and *Larix europaea*. He found that the illumination of shoots in air containing carbon dioxide caused a large increase in the subsequent respiration in the dark, this increased rate falling away to normal over a period of days. Illumination in CO_2 -free air gave no such increase, suggesting that observed effects in the presence of external CO_2 were purely ergastogeneus. Similar effects in air containing CO_2 were obtained by Detmer (1893) in seedlings of *Lupinus luteus*. Meyer and Deleano (1912) could observe no direct effect of illumination of *Helianthus tuberosus* in CO_2 -free air. Matthaei (1904) observed the stimulating effect of active assimilation periods on the subsequent respiration rate in the Cherry Laurel, and she regarded this as not being due entirely to increased substrate for respiration. Spoehr and McGee (1923) illuminated leaves of *Helianthus* on 7 per cent. glucose solution and found that there was a considerable decrease in the subsequent respiration in the dark. They attribute this effect to the destruction of amino-acids, which are supposed to have a stimulating effect on the respiration. They also found that illumination of leaves on water and in air containing CO_2 resulted in an increase in the subsequent respiration in the dark. They regard this effect not as an ergastogeneus one but due to the intimate protoplasmic coupling of respiration with assimilation. Van der Paauw (1932) also measured respiration after illumination and found that it was well above the normal value before illumination. Parija and Saran (1934) found that illumination periods increased both the respiration and the

concentration of sugars in cut starved leaves of both green and albino varieties of *Aralia* sp. They found that after 70 to 90 hours' starvation they could obtain no assimilation of externally provided CO_2 . Since their stimulation effects on the respiration were obtained, for the most part, on leaves starved for greater periods, they conclude that the action of the light is probably an activation of the respiratory enzymes or the enzymes' hydrolysing reserves.

The stimulating effects of periods of assimilation of externally provided CO_2 on the respiration of a range of green tissues seems to be definitely established, but varied results have been obtained for illuminations in CO_2 -free air. Since theories of the mechanism of the action of illumination have been based on the differences in the reactions of the plant to illuminations in CO_2 -free and CO_2 -enriched air, it seems desirable that exact comparisons of effects under these two conditions should be made. In the work to be described a wide range of illumination conditions has been explored, particular attention being paid to accurate comparisons of after-effects of illumination, under identical conditions of light and temperature, in CO_2 -free and CO_2 -enriched air. Accurate observations of the CO_2 absorption of the shoots under the latter conditions have also been made in order to obtain quantitative measures of the effect of this 'extra' assimilation. In this way it was hoped to elucidate the true nature of the effect on the respiration of green plants.

MATERIAL

The material used was shoots of Cherry Laurel obtained from five bushes all grown from cuttings from the same parent plant. A feature of these bushes, which lent itself ideally to this work, was the fact that they produced, on the lower branches, shoots on which the leaves were arranged with their laminae in one plane. These shoots could thus be fitted into thin flat chambers of minimum volume, thus ensuring uniform illumination of all the leaves and also accurate observation, by the pettenkofer technique, of rapid changes in the respiration rates. These flat shoots were very uniform in structure, and it was possible to obtain ample material from these five bushes every year. All shoots chosen possessed eight leaves and were gathered from branches 2 to 4 ft. from the ground on the east side of the bushes, to ensure the greatest uniformity possible in the material taken.

Shoots were gathered in the early afternoon and brought back to the laboratory in a closed container in a moist atmosphere. The ends of the shoots were then recut under water. Shoots were then weighed and sealed, with their cut ends in water, into the respiration chamber to be described. The whole procedure was carried out as rapidly as possible and generally took about an hour. Respiration (CO_2 output) was then followed at 22.5°C .

The drift of the respiration of detached leaves of the Cherry Laurel during starvation in the dark is well known from the researches of many workers (Blackman, 1908; Godwin, 1926; Godwin and Bishop, 1927, et al.). A typical curve that is obtained is seen in Fig. 1, where the various phases are clearly marked. The ultimate factors controlling the respiration rate in these phases

remains relatively obscure, although the problem has been intensively studied. It has been shown that the floating respiration descends to the flat slowly-falling asymptote of the protoplasmic respiration along an approximately logarithmic curve (Godwin, 1926), but this curve is not correlated with any similar fall in the starch reserve of the leaf (Deleano, 1912; Narain, 1933). Starch is depleted gradually throughout the floating and protoplasmic phases and does not disappear completely until the senescent phase. It has been found, however, from biochemical evidence (Deleano, 1912; Yemm, 1935)

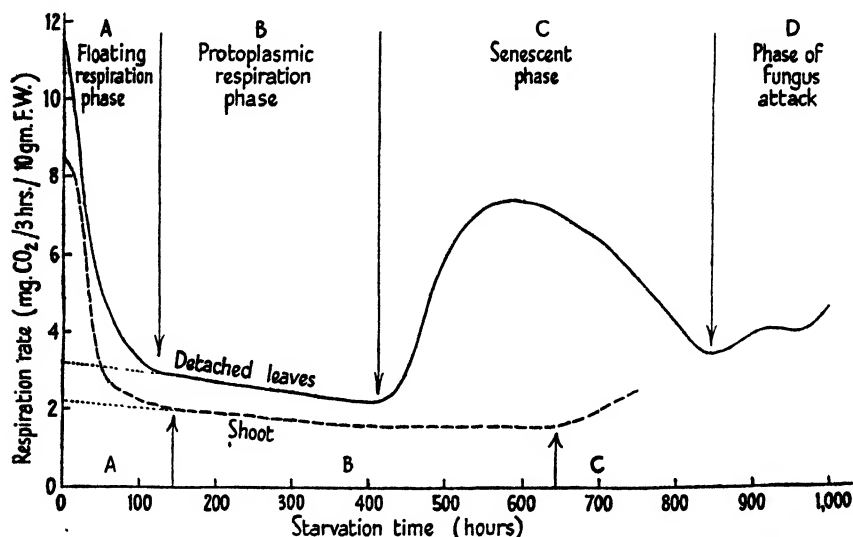


FIG. 1. Graphs showing the drifts of the respiration of detached leaves (continuous line) and a typical shoot (interrupted line) during starvation in the dark at 22.5° C.

and from measurements of the R.Q. (Godwin, 1926) that although mostly carbohydrates are respired, yet an additional substrate of the nature of protein is respired in the protoplasmic phase. It seems therefore that we are dealing with at least two sources of carbon for respiration, and that the observed curve is the result of the changing activity of one or more of the various enzyme systems associated with respiration, rather than with changes in the concentration levels of substrates. Thus traumatic stimulation of leaves during gathering from the bush may play an important part in setting the high levels of respiration in the floating phase (Audus, 1935). In the symmetrical senescent hump of the respiration there is an increase in the rate of consumption of all the respiratory substrates (Godwin, 1926; Godwin and Bishop, 1927) due to the lowered protoplasmic organization resistance arising from protein depletion in the protoplasmic phase.

The drift of the respiration (CO₂ output) during starvation of shoots of the Cherry Laurel is very similar to that of detached leaves, but differs in one or two important respects. The drift for a typical shoot is also shown in Fig. 1. A large number of experiments by the author have shown that the character-

istics of the floating respiration phase of shoots are identical with those of detached leaves. Although the pitches and rates of fall of respiration in the protoplasmic phase are similar, yet in shoots the duration is much longer. Thus senescence is considerably delayed and, when it does occur, is much more gradual, giving a much elongated and flattened 'hump'. The cause of the delayed senescence is obscure.

This long protoplasmic respiration phase of shoots with its very slowly falling pitch enables a relatively extensive series of experiments to be carried out over a period when the respiratory system is presumably undergoing at most a slight change, thus eliminating the complications which would be produced by the changing conditions of the floating and senescent phases.

EXPERIMENTAL DETAILS

The method employed in the following researches was essentially an elaboration of the technique of Borodin. Shoots were allowed to respire in the dark with their cut ends in water at a constant temperature and then, at definite intervals in their starvation life, they were subjected to periods of illumination, either in CO_2 -free air or in air containing a known concentration of CO_2 . The subsequent respiration of shoots in the dark was followed. It was hoped that a complete analysis of the after-effects might throw light on the course of the respiration during the illumination period. With very few exceptions, all experiments have been carried out on the protoplasmic respiration phase.

The chamber used in these experiments is illustrated in Fig. 2. The thin flat compartment was formed by the ebonite back-plate A, the 5-mm. glass front-plate, and the ebonite frame B, giving the internal dimensions of 14 in. \times 10 in. \times $\frac{1}{4}$ in. and a volume of half a litre. Watertight joints were ensured by carefully ground plane surfaces of contact of glass and ebonite smeared with vaseline. Gas current, water-supply, &c., inlets were all through the ebonite back-plate. Setting up and dismantling of the chamber was easy and rapid by making use of milled-edged nuts on the bolts of the clamping frame C.

The cut end of the shoot was placed in a small silvered dish of about 2 c.c. capacity, provided with a side-tube (Fig. 2), passing through a bung in the lower of the two large holes and attached to a large vertical glass tube fitted at the lower end with a glass cock. By this arrangement the small silver dish could be kept filled with water during an experiment of many weeks' duration.

Respiration was followed in the dark at a temperature of 22.5°C . A steady current of CO_2 -free air was maintained through the chamber at a rate of 2 litres per hour. The air was drawn from the outside of the laboratory to eliminate any effects of ethylene contamination from laboratory air. Continuous 3-hourly readings of the respiration were taken by using the Blackman automatic air-commutator arrangement. The air stream issuing from the chambers was bubbled through 75 c.c. of N/15 barium hydroxide solution in pettenkofer tubes. Estimations of the amount of CO_2 absorbed were carried out by back titration with N/10 HCl.

With the exception of two preliminary experiments all illuminations have been carried out by artificial illumination, provided by tungsten filament lamps of various powers and arranged at various distances from the chambers. To reduce the heating effects of these lamps, the whole of the glass bulbs were immersed in flowing water in inverted bell-jars. In the majority of

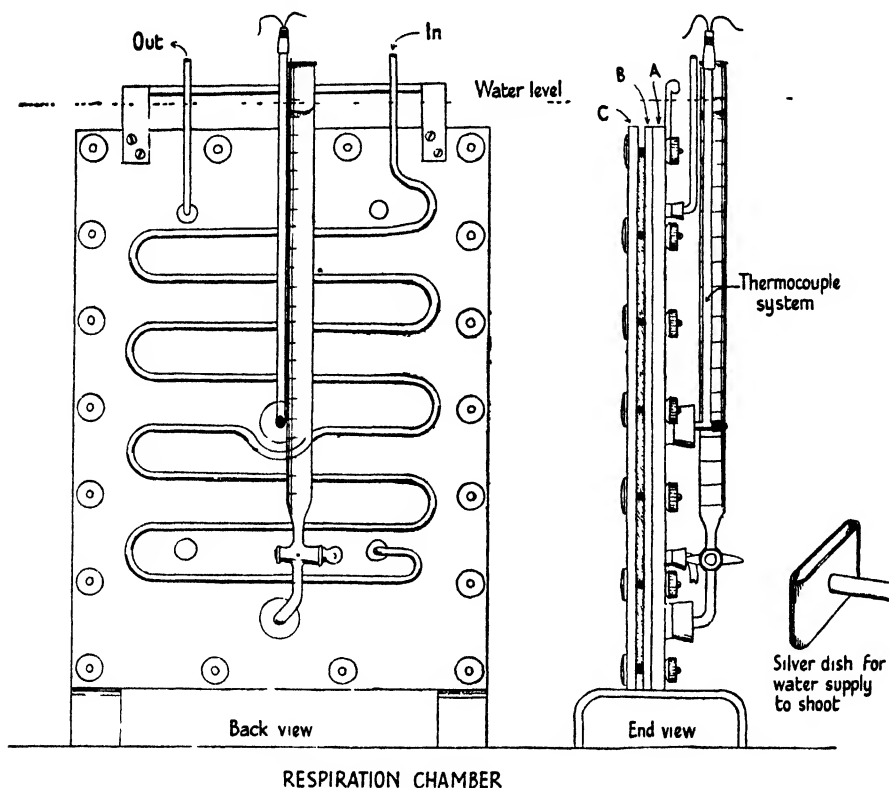


FIG. 2. Diagrams showing the construction of the respiration chamber used in the illumination experiments.

experiments (IV to XVI inclusive) the respiration chambers themselves were immersed during the illumination in a glass tank filled with running water. This water was obtained from a large roof tank, and remained at a temperature which did not fluctuate more than half a degree Centigrade during the whole of an illumination experiment. After the illumination the chambers were replaced in the thermostat at 22.5°C . and the respiration followed at that temperature.

Different intensities of artificial illumination were obtained with lamps of 250, 500, 1,000, and 1,500 watts arranged at a distance of 25 cm. from the shoots. The lamp of 1,500 watts gave approximately 3,000 candle-power. In order to produce higher and intermediate intensities the lamps were covered, except for a small window next to the chambers, with aluminium

paint, internal reflection from this increasing the intensity by about 30 per cent. The average intensity of total radiation produced by each lamp over the shoot was measured by a sensitive Moll thermopile and moving-coil galvanometer.

It is a well-known fact that illumination of a leaf in a closed space, where transpiration is impeded, causes a considerable rise in the temperature of the leaf over its surroundings. During illumination therefore measurements have been made of the average temperature of the leaf over its surroundings. An apparatus was constructed consisting of four delicate copper-constantan thermo-couples arranged in series. This system of couples was sealed into a glass T-tube, one arm of which passed through the central bung in the back-plate of the respiration chamber. The four warm junctions were in the form of very fine needles, which could be inserted for a considerable distance into the mesophyll of the leaf. The four cold junctions projected a short way out into the bath in which the chambers were immersed. A mercury thermometer was used to measure the temperature of these junctions. By measuring the total E.M.F. over the couples by a sensitive galvanometer, previously calibrated for a known temperature difference, the average temperature of four out of the eight leaves on a shoot could be found at any time during the illumination. It was found that leaves thus illuminated reached a steady temperature above that of the water in the bath after about 15 minutes. This temperature difference was directly proportional to the total radiation intensity over the leaf, and was approximately 4.5°C . for the highest light intensity used (1,500-watt lamp at 25 cm.). The temperature of the water surrounding the chambers during illumination varied slightly from one experiment to another. Thus in experiments of the highest light intensity the temperature of the shoots during illumination was approximately that at which respiration was measured. In other experiments, however, with lower light intensities the temperatures were somewhat lower. Account of these temperature differences have been taken and are referred to in the analysis of results. It should be noted that the temperature of the water in the glass tank and thus that of the leaves could be adjusted within narrow limits by regulating the rate of flow through the tank. In any series of illumination experiments on any one shoot, care was taken to adjust this temperature to approximately the same value during each illumination, allowing therefore of a rigid comparison of effects within the series.

In the first two experimental series (I and III) the shoots were illuminated in still air, in chambers which were sealed by water-bulb manometers for equalizing the pressure between the inside and the outside. Two experiments were run simultaneously, one shoot being illuminated in CO_2 -free air and the other in air enriched in CO_2 . In the latter case a known quantity of pure CO_2 was introduced into the chamber just before the illumination period. The amount of CO_2 absorbed by the leaves from the surrounding atmosphere during the experiment was found by estimating the amount of CO_2 remaining over in the chamber after the illumination. To do this the chamber was

swept out rapidly by a current of air and the CO_2 absorbed in barium hydroxide solution. In all other experimental series, with the one exception of XV, illumination of shoots was carried out with a current of air of 2 litres per hour passing through the chambers. In the cases where two parallel experiments, similar to the above, have been carried out, one stream has been of CO_2 -free air and the other of air containing a known concentration of CO_2 . This was admitted at a constant rate to the gas stream by bubbling through NaHCO_3 solution into which HCl of known concentration was allowed to drop at a constant rate. The NaHCO_3 solution was also of known volume and concentration. The CO_2 issuing from the chamber during the illumination was absorbed in $\text{N}/2$ NaOH in pettenkofer tubes. This amount of CO_2 was estimated by a double titration technique in a closed system. This precaution was necessary since it has been found (El Gawadi, 1935) that the titration of a mixture of hydroxide and bicarbonate with strong acids invariably results in a loss of CO_2 from the system. El Gawadi's method was used and consisted of neutralizing the excess NaOH with HCl in a closed system using phenolphthalein as an indicator. The BaCO_3 was then estimated by further titration with HCl using methyl orange as an indicator. A similar technique was used to estimate the amount of NaHCO_3 remaining in the bottle. An estimation of excess CO_2 remaining in the chamber at the end of the illumination was necessary before the amount absorbed by the shoot could be determined. As a result of this excess the reading of the apparent respiration after the illumination was much higher than could possibly be accounted for by the true respiration of the shoot. This excess CO_2 was estimated in the following way. The true respiration for the first 3 hours after illumination was estimated by extrapolating the subsequent readings of the effect. This gave a probable value of the true initial respiration with an error of not more than a few per cent. Any observed CO_2 over and above this value was regarded as non-respiratory CO_2 from the gas stream. From these three estimations of the excess CO_2 the total amount of assimilation could be found by difference from the total amount in the original bicarbonate solution. The only inaccuracy involved in this estimation was that in the value of true respiration of the shoot for the first 3 hours after the illumination. In view of the high rates of assimilation relative to respiration this error is negligible. In the majority of experiments the concentration of CO_2 in this air stream was approximately 5 per cent.

THE AFTER-EFFECTS OF ILLUMINATION

Preliminary experiments (experiment series I)

This first experiment series was carried out on a pair of similar shoots, and was entirely of an exploratory nature. Little attention was therefore paid to the accurate determination of assimilation rates, leaf temperatures during illumination, &c. The results will be briefly described, however, to serve as an introduction to the more carefully planned experiments which constitute the bulk of the work.

The first illumination was carried out, after 117 hours' starvation in the dark, by direct midday sunlight for 2.5 hours. Both chambers were sealed and one contained 10 c.c. of pure CO_2 introduced immediately before the start of the illumination. No estimate of leaf temperatures during illumination was made, but it is probable that it rose to about 35°C . In Fig. 3 we see

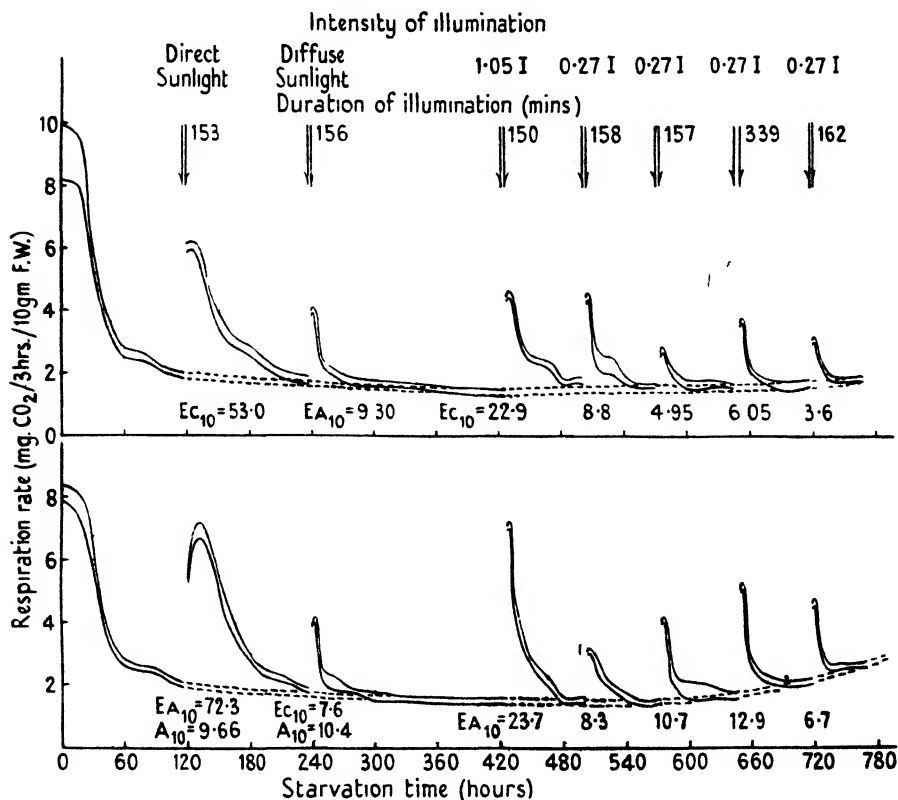


FIG. 3. Graphs showing the complete smoothed respiration curves for experiment series I, together with details of conditions of illumination for each experiment and the size of each illumination effect. The double lines represent the limits of scatter of individual 3-hour readings.

the effect of this procedure on the subsequent respiration, i.e. a very considerable stimulation in both shoots. The maximum respiration rate is reached very soon after the cessation of the illumination, and falls away gradually to normal over a period of about 100 hours.

In the computation of results the total extra CO_2 emerging in the effect, over and above the normal drift of the respiration, has been taken as a measure of the effect. This was obtained by finding the area of the graph between the smoothed curve of observed respiration and the normal interpolated drift (mean of dotted lines in the graphs of Fig. 3). This measure of the effects, in terms of total amounts of CO_2 in milligrams per shoot, is recorded in Fig. 3.

In order to simplify subsequent descriptions these values have been given the symbols Ea , for the results of illumination in CO_2 -enriched air, and Ec , for illuminations in CO_2 -free air. When calculated per 10 gm. fresh weight of shoot, the symbols which will be used are Ea_{10} and Ec_{10} respectively. These latter values correct for differences due to different weights of the two shoots used, and allow direct comparisons of effects. In this experiment it will be seen that Ea_{10} is larger than Ec_{10} . The possible significance of this will be discussed later.

It is quite clear that the illumination of shoots in direct sunlight for 2.5 hours produced large effects on the subsequent respiration rate in the dark. After the respiration had again reached normal, a second illumination experiment was carried out as before, but here the illumination was the diffuse light of an overcast noon. In this case, the air surrounding the second shoot was enriched with 10 c.c. of pure CO_2 . Results are shown in Fig. 3. It will be seen that, even in this much-reduced illumination intensity, exposures of 2.5 hours caused a considerable rise in the subsequent respiration and that, here again, although conditions of CO_2 supply had been reversed for the two shoots, Ea_{10} is larger than Ec_{10} . The return of the respiration to normal takes place in rather a shorter time than in the previous effect. It seemed quite probable that the difference in magnitude between these two pairs of effects was due to differences in illumination intensity. The remaining illuminations in the series were therefore carried out with artificial light. The complete respiration curves for the whole series are shown in Fig. 3, with data for the range of illumination conditions. The light intensity in the latter illuminations have been expressed in terms of an arbitrary unit I , which was the intensity produced by a 1,500-watt lamp at 26 cm. The visible radiation intensity corresponding to I was approximately 48,000 lux. The graph shows that an intensity of 0.27 I produced a considerable rise in the respiration rate and, owing to its comparatively small heating effect, this intensity was chosen for the next experiment series (III).

An interesting fact that appears from these results is that, with one exception, the values of Ea_{10} are larger than the corresponding values of Ec_{10} . It seemed likely that this difference was due, directly or indirectly, to the assimilation of CO_2 provided externally to the shoot. This total CO_2 absorption (subsequently referred to as A , and when calculated per 10 gm. fresh weight of shoot as A_{10}) has been determined in all of the above experiments and is plotted in Fig. 4, I. In this graph the vertical scale is the average rate of apparent assimilation in gm. of CO_2 per hour per sq. metre of leaf area. The horizontal scale of the rectangle bases is the time of duration of the assimilation in hours. The area of the rectangles therefore give a measure of the amount of externally supplied CO_2 assimilated per unit area of leaf during the whole of the illumination. The values of $Ea_{10}-Ec_{10}$ have also been determined and their ratios to the corresponding values of A_{10} found for each experiment. These ratios appear by the side of the corresponding rectangles in the figure and have also been plotted as black fractions of the assimilation

rectangles. It will be seen that with one exception these ratios are positive, but show a large range. In the case of the first illumination in sunlight the

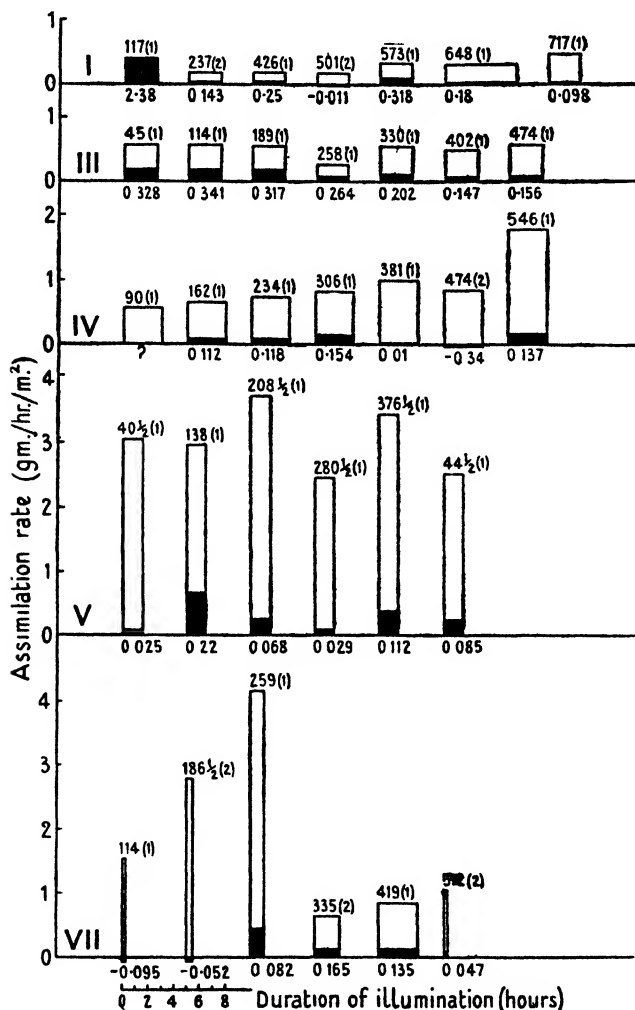


FIG. 4. Diagram showing the collected results of illuminations in CO₂-enriched air in experiment series I, III, IV, V, and VII. The large rectangles represent the total amount of CO₂ assimilated by the shoot per square metre of leaf area during each period. The figures above each rectangle record the starvation time at the commencement of the corresponding illumination period, and the small ringed figures the shoot illuminated in CO₂-enriched air. The figures at the base of the rectangles record the corresponding values of $(E_{a10} - E_{c10})/A_{10}$, this ratio being further illustrated by being drawn as a black fraction of the large rectangle.

value obtained is 2.38. In no other experiment has a value of over 1.0 been reached, and it has been concluded that this relatively high ratio is due to shoot variation made apparent in the exceptionally large effects of illumination in direct sunlight. The significance of the other ratios will be considered later.

Drifts of effects with starvation (experiment series III)

Having, in the preliminary experiment series, explored to a certain extent the range of conditions causing an effect of measurable size, it was decided to carry out an investigation of the drift of effects under constant conditions of illumination throughout the starvation life of the shoot. The results of this series of illuminations on one pair of shoots, and also the details of illumination conditions, are shown in Figs. 5 and 4, III. The chambers were sealed

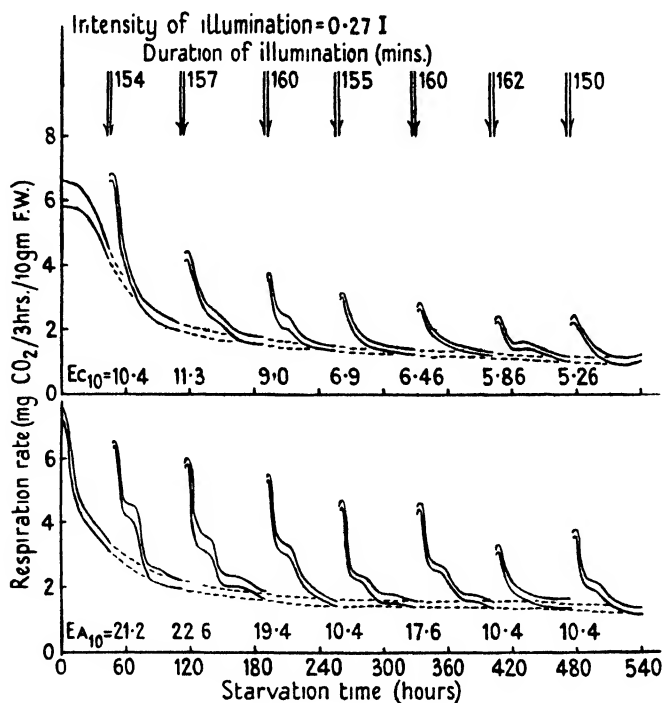


FIG. 5. Graphs showing the complete smoothed respiration curves of experiment series III, together with the details of conditions of illumination for each experiment and the size of the illumination effects. Construction of graphs as in Fig. 3.

during the illuminations and 40 c.c. of CO₂ introduced into chamber 1 immediately before each illumination. In this series the temperature of the air inside the chambers during the illumination was found by means of a single thermocouple placed behind a leaf.

If we consider the effects of illumination in CO₂-free air we see that they form a very uniform series, descending gradually in magnitude as starvation proceeds. In Fig. 6 the values of Ec are plotted against the corresponding values of normal respiration drift level just before illumination. With the exception of an effect on the floating phase of the drift, there is a striking linear relationship between these two sets of values.¹ It is possible that during

¹ Subsequent work has shown that the effects of illumination on the floating phase are exceptions to the generalizations that have been drawn concerning effects on the proto-

illumination the respiration is put up to a value bearing a constant ratio to that in the dark. All the respired CO_2 is assimilated and piled up in the leaf in the form of some photosynthetic product. The observed effect curve might then represent the exhaustion of these accumulated substrates. The total CO_2 of the effect (E_c) would therefore be equal to the respiratory CO_2 assimilated during the illumination. If this is the case, then the duration of the illumination should enter into the picture, and the more prolonged the illumination the greater should be the value of E_c . In this series, however,

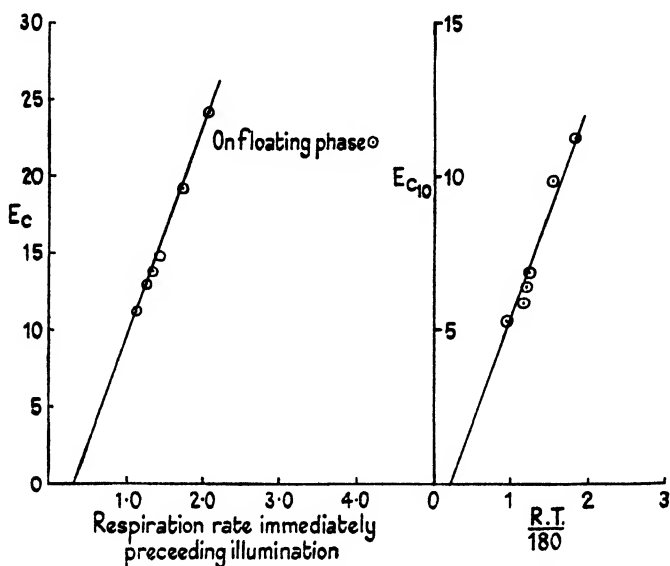


FIG. 6. Analysis of the effects of illumination in CO_2 -free air in experiment series III. Graphs showing the relationship of size of effect (E_c and E_{c10}) to the normal respiration intensity (R) and the duration of the illumination (T).

the duration of the illumination remained relatively constant, and we had therefore the observed linearity. In order to see whether corrections for this possible time effect would make a closer fit of observed points to linearity, the values for the following expression for this shoot were calculated for each illumination experiment: $RT/180$, where R = the respiration intensity immediately before the illumination (from the smoothed drift curves) in mgs. CO_2 per 10 gm. fresh weight of shoot per 3 hours, and T = the duration of the illumination in minutes.

The above expression is equal to the total amount of CO_2 per 10 gm. fresh weight of shoot that would have been respired in the dark at 22.5°C . during the illumination period. This gives, therefore, if the above assumptions are correct, a measure of the amount of assimilates piled up in the shoot. In the right-hand graph of Fig. 6 this expression has been plotted against the

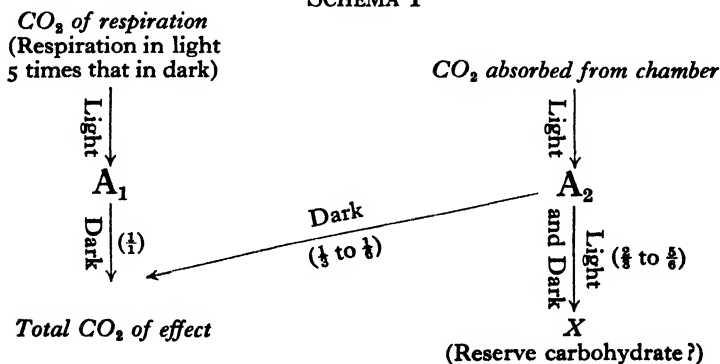
plasmic phase of the starvation drift. In subsequent experiment series of this type illuminations have been confined to the protoplasmic respiration phase.

corresponding values of Ec_{10} . The value for the effect on the floating phase of the drift has been omitted. It will be seen that the corrected values do not fit closer to the linear relationship than the non-corrected values. Later experiments, however, with a large range of duration of illumination, show that this time factor does play a part.

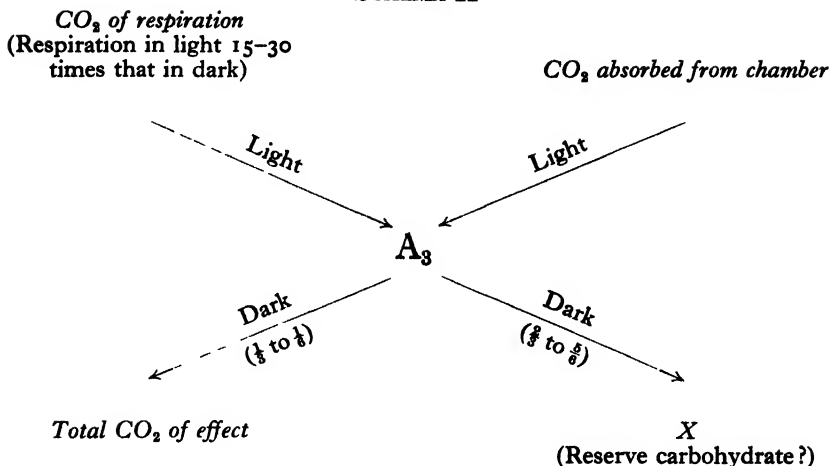
If we turn our attention to the effects of illumination in an atmosphere enriched in CO_2 , we see that they also form a uniform series, Ea being consistently greater than the corresponding value of Ec . These results are even more striking when the values of $(Ea_{10} - Ec_{10})/A_{10}$ are compared. These ratios, which are seen beside the corresponding assimilation rectangles in Fig. 4, III, show a downward trend with time of starvation of the shoot, starting in the early stages with a value of approximately $1/3$ and falling to about $1/6$ after 500 hours. Thus, if the observed differences in effects are due to the extra assimilation carried out by the shoot in CO_2 -enriched air, then never more than a third of that CO_2 comes out again in the subsequent respiration of the effect. It is, of course, conceivable that the observed difference might be a property of the particular pair of shoots used. In order to eliminate this possibility each shoot should be subjected alternately to illumination in CO_2 -free and CO_2 -enriched air. This has been done in some subsequent experiment series. One fact, however, that tends to support the first explanation is the result of the experiment carried out after 256 hours of starvation. In this case the amount of extra assimilation, A , was half that in all the other experiments, but it will be seen that $Ea_{10} - Ec_{10}$ for this effect is correspondingly decreased, giving a value of $Ea_{10} - Ec_{10}/A_{10}$, which fits closely onto the above-mentioned descending series.

It seems probable therefore that the increase in effect in CO_2 -enriched air is due to 'extra' assimilation but that this increase, in terms of total CO_2 exchange, is only 15 to 30 per cent. of this total 'extra' assimilation. If therefore we explain the effects of illumination in CO_2 -free and CO_2 -enriched air as due to piling up of assimilates in the leaf, we have the following two possible explanations of the observed facts of III. The two following schemata serve to give a pictorial idea of the two possibilities.

SCHEMA I



SCHEMA II



In this first schema, by illumination in CO_2 -free air respiratory CO_2 is photosynthesized to assimilate A_1 which is completely respired in the subsequent effect. It has been seen from Fig. 6 that the total amount of CO_2 of the effect is approximately five times the amount of CO_2 that would have been produced by normal respiration over the illumination period. We must therefore assume on this hypothesis that the average respiration in the light is five times the normal drift in the dark. In CO_2 -enriched air the assimilated respiratory CO_2 has the same fate as in CO_2 -free air, but the CO_2 provided externally is photosynthesized to a different assimilate A_2 . In the subsequent effect only a third to a sixth of this assimilate is respired, the rest being anabolized to reserve carbohydrate X .

In schema II the assimilation of both respiratory CO_2 and CO_2 provided externally gives rise to a single assimilate A_3 . In the dark only a third to a sixth of this total assimilate reappears as the CO_2 of the effect, the rest being anabolized, as above, to reserve X . It follows from this schema that to give the observed results of III the average respiration in the light must be 15 to 30 times that in the dark.

Improbable assumptions enter into both these schemata. In the first it is very unlikely that there are two distinct assimilates from two different CO_2 sources. In the second it is improbable that the average respiration in the light is 15 to 30 times that in the dark, since in this case we should expect a respiration rate approximating to this in the first dark period after the illumination. It therefore seems likely that the immediate causes of the observed effects do not lie in an increased concentration of respirable substrate in the form of newly produced assimilatory products. In other words the effect is a plasmogeneous one. This theory, together with other possibilities, will be discussed later with the results of other experimental series.

The relationship of E_c to R and T

In the last section it has been seen that, within a narrow range of values of T , the results of experimental series III conformed very closely to the expression

$$E_{c10} = \frac{\alpha RT}{180},$$

where α is a constant for the particular set of lighting conditions in the series, &c. A number of subsequent experiment series have been carried out in which this expression has been tested with a very wide range of duration of illumination.

The first series of experiments on this problem was VII. Here two similar shoots were used, and parallel illuminations carried out in streams of CO_2 -free and CO_2 -enriched air. These treatments were alternated for each shoot, the illumination intensity being the same for each experiment. The values of E_{c10} obtained have been plotted against the corresponding values of $RT/180$ in the first graph of Fig. 7 (ringed points). With this very extended range of T (15 to 174 minutes), results indicate that the relationship is not strictly linear, the curve bending towards the $RT/180$ axis as that value is increased. Owing to the fact that the illuminations were carried out at a lower temperature than the respiration in the dark, corrections have been made for this temperature difference in the values of R , which is an estimate of the basic respiration rate in the light. The adjusted value of $RT/180$ is therefore the amount of CO_2 which would have been respired by 10 gm. of the shoot in the dark over the illumination period and at the illumination temperature, assuming a Q_{10} of 2 for respiration over this temperature range. These corrected values appear as black points in the graph. These corrections make no significant change in the nature of the relationship.

In experiment series XIII a still greater range of duration of illumination was used (23 minutes to 9 hours), the intensity again being kept constant for all illuminations in the series. A single shoot was used, and illuminations were all in CO_2 -free air. Analysis of this series is seen in the second graph of Fig. 7. For low values of $RT/180$ it will be seen that the points fall on a straight line, but with the higher values there is considerable scatter. It is probable that the true relationship is shown by the curve in the graph. This is borne out by the results of XV.

In this experiment series two shoots were used, one being illuminated in a current of CO_2 -free air and the other subjected to the same illumination conditions in still CO_2 -free air, the chamber being sealed. These treatments were alternated, the illumination intensity remaining constant for all experiments in the series. The range of duration of illumination was further extended (71 minutes to 12 hours). The results are seen in the third graph of Fig. 7. The following points follow from the graphs: (1) the expected bending of the curve towards the $RT/180$ axis is verified; (2) the results from the two shoots form two distinct curves, showing the variability to be expected

between shoots; (3) no significant difference due to conditions of air flow during illumination.

In experiment series XVI a similar extended range of duration of illumination was investigated in two different light intensities of 1 and 0.132 I arbitrary units, all other conditions being constant for the series. The last graph

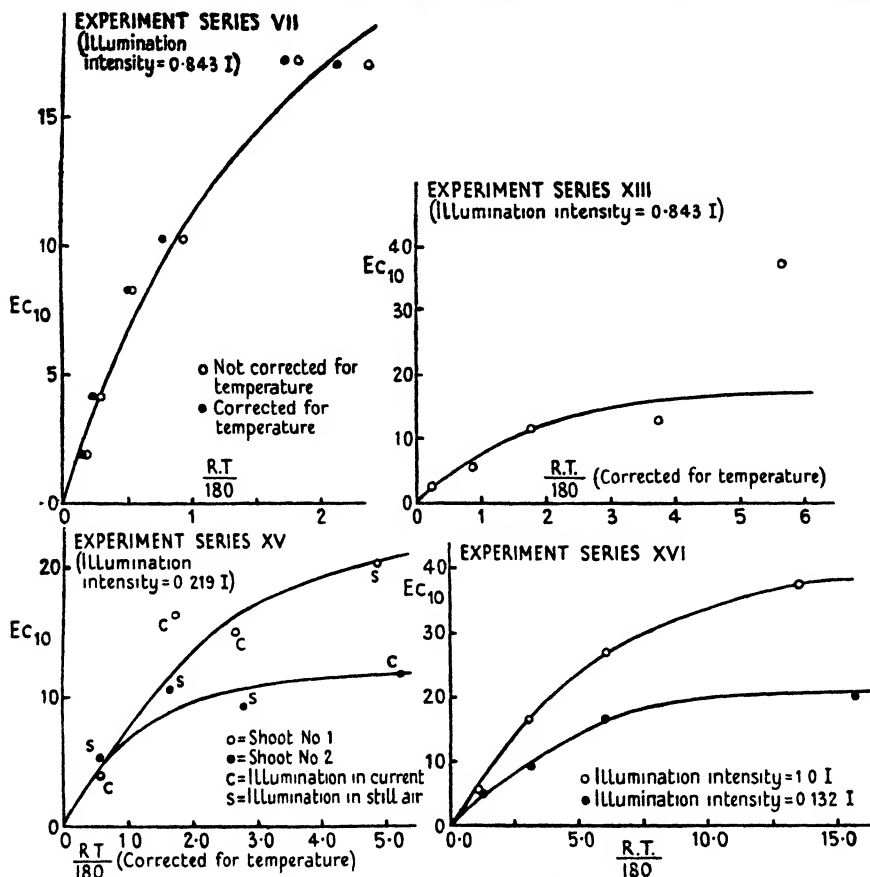


FIG. 7. Analysis of the effects of illumination in CO_2 -free air in experiment series VII, XIII, XV, and XVI. Graphs showing the relationship of the size of the effect (Ec_{10}) to the normal respiration intensity (R) and the duration of illumination (T).

of Fig. 7 shows the results of this series. Thus for both illumination intensities the curves have a form similar to those obtained from previous series. In addition, the values of Ec_{10} for the high light intensities are approximately twice as large as those for the low light intensities. The ratio of the light intensities is, however, approximately 8:1. It seems therefore that the size of the effect is not directly proportional to the light intensity.

The effect of light intensity

One complete experiment series has been performed on one shoot to investigate the relationship of effect to illumination intensity. In this

series, XII, illuminations were carried out in a stream of CO_2 -free air. A wide range of illumination intensities was used and distributed at random throughout the various illumination periods of the series. In order to eliminate from the values of Ec_{10} any variations due to different values of $RT/180$, the duration of any illumination was given a value such that $RT/180$ was kept a constant throughout the whole series. In this way later illuminations were longer than earlier ones in the series. Results are seen in Fig. 8, where

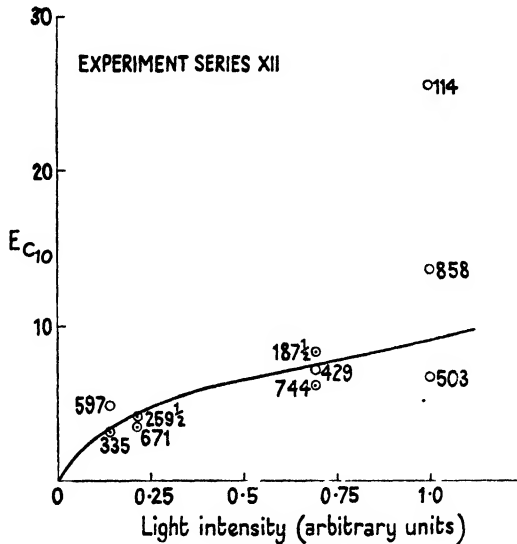


FIG. 8. Analysis of the effects of illumination in CO_2 -free air in experiment series XII. Graphs showing the relationship between the size of the effect (Ec_{10}) and the illumination intensity in arbitrary units ($I = 48,000$ lux approximately).

the values of Ec_{10} are plotted against those of the light intensity. The numbers against the points are the starvation times of the shoot, in hours, at which the corresponding illuminations were started. It will be seen that Ec_{10} increases as we increase the light intensity, a result forecast in previously mentioned results (series I and XVI). There is, however, a large scatter of values, especially in the case of the high light intensities, and this scatter is not correlated with the time at which the experiment was carried out.

The relationship between Ea_{10} , Ec_{10} , and A_{10} . The ratio $(Ea_{10} - Ec_{10})/A_{10}$

The results of experiment series III have shown that, as a general rule, in illuminations carried out under identical conditions, the value of Ea_{10} is greater than that of Ec_{10} , and that the ratio of the difference of these two quantities to the amount of 'extra' assimilation per 10 gm. fresh weight of shoot shows a steady downward drift with starvation of the shoot, and falls between the values of 0.33 and 0.15. The results of series I were not uniform, and this has been attributed to the fact that the illuminations were carried out under a variety of conditions.

A number of other experiment series have been performed to investigate this ratio still further. The results of experiment of this nature are collected together in Fig. 4.

In experiment series I and III a known quantity of CO_2 had been introduced into the chamber which was sealed during the illumination. In this way the CO_2 concentration in the chamber was constantly falling during the course of the illumination. In subsequent series illuminations have been carried out in a stream of air with constant concentration of CO_2 .

In series IV various intensities of illumination were used to investigate the effect of increasing the value of A_{10} . In the first four illuminations shoot 1 received the air current enriched in CO_2 , while shoot 2 acted as control in CO_2 -free air. In the fifth illumination these conditions were reversed. By this means it was hoped to throw light on any intrinsic differences that might exist between the two shoots. In the last illumination conditions were the same as in the first. It will be seen from the ratios $(Ea_{10} - Ec_{10})/A_{10}$ that no simple relationship exists between the differences in the two effects and the amount of 'extra' assimilation. The value of this ratio for the fifth illumination is actually negative, i.e. the effect for shoot 1 remained greater than that for shoot 2, in spite of the reversed conditions of CO_2 supply. The pitch of the respiration drift of shoot 1 was, however, higher than that of shoot 2 (see next section). It is therefore possible that the consistently greater effects from shoot 1 are mainly correlations with the greater value of R for that shoot. In this case the value of $\frac{Ea_{10}}{RT/180}$ for any illumination should not be significantly different from the corresponding value of $\frac{Ec_{10}}{RT/180}$. In Table I these two sets of values have been calculated from the results of series IV.

TABLE I

Illumination (min.).	$\frac{Ea_{10}}{RT/180}$	$\frac{Ec_{10}}{RT/180}$	No. of shoot illuminated in CO_2 -enriched air.
162	10.53	5.25	1
234	13.88	8.56	1
306	14.76	7.26	1
381	12.72	10.49	1
474	11.20	8.74	2
546	19.62	10.28	1

It will be seen from this table that the first ratio is consistently greater than the second, even in the case of the fifth illumination under the reversed conditions of CO_2 supply, thus suggesting that the observed differences are not due to errors of random sampling in the two shoots chosen, but real and due to 'extra' assimilation.

In experiment series V assimilation rates were pushed still higher by using a much higher concentration of CO_2 in the gas stream (5 per cent.). The illumination periods were of approximately half the duration of those in series

IV, and the illumination intensity remained constant for all the experiments. Here again the values of $(Ea_{10}-Ec_{10})/A_{10}$ show great fluctuations, but do not differ significantly from the ratios obtained in previous experiments.

The results of series VII are even more erratic. Thus in two out of six experiments Ec_{10} was greater than the corresponding value of Ea_{10} . It will be observed that these two effects were for illuminations of short duration. These illuminations gave small effects, and, as a result, the errors involved in the estimation of Ea_{10} and Ec_{10} were of the same order as expected differences between them. Thus not much reliance can be placed upon these small measured differences. In the three larger effects the ratio is of the same order as those from other series.

It seems safe to conclude from the foregoing results that illumination of shoots in an atmosphere enriched in CO_2 produced a slightly greater after effect than illumination under similar conditions in CO_2 -free air. It is difficult to see what relationship the magnitude of this difference bears to such variables as the amount of 'extra' assimilation, time of starvation at which the experiment was carried out, &c.

THE EFFECT OF THE ASSIMILATION OF EXTERNALLY PROVIDED CO_2 ON THE STARVATION DRIFT LEVELS

In addition to the immediate effects of 'extra' assimilation in causing a slight increase in the subsequent after-effect, there is also a marked effect on the pitch of the starvation drift level of the respiration. In all cases, continued periods of illumination in CO_2 -enriched air gave rise to higher rates of drift respiration than continued periods in CO_2 -free air. Again, the greater the total amount of assimilation carried out by the shoot, the greater the difference between the two drift rates. Thus in series I and VII, where treatment received by the two shoots was alternated, and both shoots received approximately the same amount of 'extra' assimilated CO_2 , the two drifts follow each other very closely. In other experiment series, where the treatments were not alternated, there is a difference in the pitch of the two drifts, which show a positive correlation with the total amount of 'extra' assimilation carried out by the one shoot. These above facts suggest that this effect of the assimilation of externally provided CO_2 is due, directly or indirectly, to the difference in content of carbohydrate reserve brought into being by the assimilation. This cannot be subjected to a direct test, but calculations have been made of the total CO_2 loss or gain of the shoots from the commencement of starvation to the commencement of each illumination period. It has been found that the difference of pitches of the normal respiration in any pair of shoots at the commencement of the illumination periods is directly correlated with the corresponding calculated differences in the total CO_2 loss. The results of analyses of series III, IV, and V are seen in Fig. 9. These correlations add considerable support to the above theory.

THE EVOLUTION OF CO_2 BY THE SHOOT DURING ILLUMINATION IN CO_2 -FREE AIR

In the series VII, XII, and XIII observations of CO_2 evolution were not confined to the dark periods. Careful measurements were also made of the rate of CO_2 evolution of the shoot during illumination in CO_2 -free air. It was found that in all the illumination intensities investigated CO_2 continued to be evolved in the light, although at a much-reduced rate.

Part of the CO_2 issuing from the chamber in the light was CO_2 of normal respiration remaining behind in the chamber and swept out in the light.

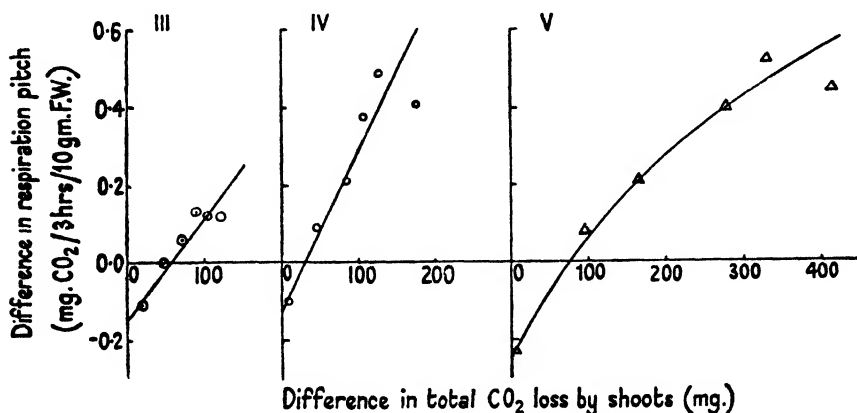


FIG. 9. Graphs showing the relationship between the difference in the pitch of the respiration drift and the total CO_2 loss of pairs of shoots in experiment series III, IV, and V.

This amount of CO_2 , which can be estimated from the results of a series of illuminations of different durations, was found to be of the order of 0.25 mg.

In order to eliminate any possibility of small CO_2 leakages vitiating the results of these experiments, in series XV and XVI great care was taken to ensure that the air entering the chamber during the illumination was completely CO_2 -free, by the use of larger and more efficient absorbing systems. Blank experiments were carried out by drawing this air through an empty chamber for many hours. No detectable CO_2 came through the system.

In these two series, as in series XIII, extended periods of illumination were investigated, and in some cases four successive 3-hour periods were followed. It is obvious that, in this way, the normal respiratory CO_2 left over from the previous dark period will be eliminated from all periods but the first. The results of these two series of experiments, together with those of series XIII, are seen in Fig. 10. Here the rates of CO_2 evolution are plotted (in mg. per 3 hours per 10 gm. fresh weight of shoot) as drifts during the illumination period. It will be observed that the value for the first period in any one set of results tend to be higher than the subsequent values, due to the inclusion of the normal respiratory CO_2 from the previous dark period. These subsequent

values show no significant drift with the time of illumination and, with one exception, fall between 0 and 0.4. It will also be observed that, although illumination intensities were different in the series of the four shoots, yet there seems to be no relationship between the observed rates and these intensities. The ratio of these rates in the light to the normal respiration rate in the dark have been calculated, for all 3-hourly periods after the first, in these experiment series XIII, XV, and XVI. In all, fifteen such ratios have

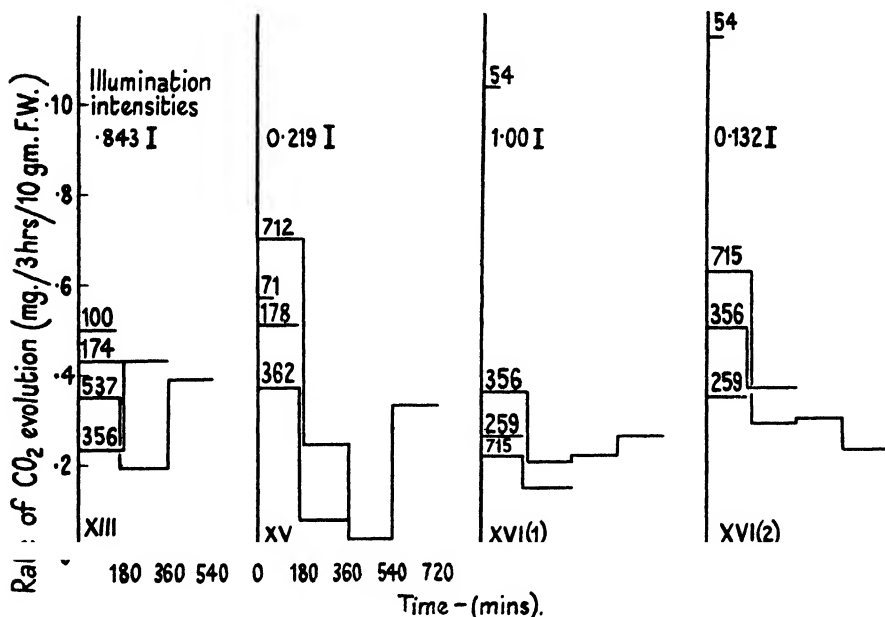


FIG. 10. Graph showing the drift of the CO_2 evolution of shoots in the light in experiment series XIII, XV, and XVI. The drifts in all the periods for any one shoot all start from the same zero in the graphs, and the total duration of the illumination is marked over the first reading of the drift. Single readings for short periods are also included.

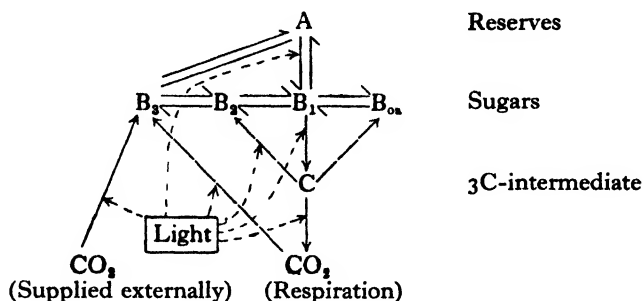
been obtained. The mean of these fifteen values is 0.089 with a standard error of 0.0146. This standard error is due almost entirely to titration error, since it has been calculated that these alone would give a standard error of the above mean of approximately 0.01. The concentration of CO_2 in the atmosphere surrounding the leaf resulting from this is of the order of from $\frac{1}{4}$ to $\frac{1}{8}$ that in the air.

This failure of the leaf to utilize all its respiratory CO_2 by assimilation in the light can be plausibly explained by the fact that the assimilatory and main respiratory centres are separated by finite distances in the cell. This fact necessitates the diffusion of CO_2 from the main respiratory centres to the assimilatory centres before assimilation can take place. Thus the ratio of CO_2 assimilated to that evolved from the leaf becomes merely a question of the relative effect of the two sinks of respiratory CO_2 , i.e. the assimilatory centre and the outside surroundings of the leaf. The larger the distance

between the two centres the greater will be the outward diffusion of CO_2 in the light.

THEORETICAL DISCUSSION AND ANALYSIS OF RESULTS

In order to understand clearly the various possibilities of conditions existing in the leaf during illumination, the following scheme has been constructed to show the fluxes of carbohydrates and the possible factors controlling them.



A, the respiratory reserve component of the leaf, need not necessarily be a single carbohydrate, but may probably represent a range of different carbon sources. In the dark this is supposed to break down by hydrolysis into sugar *B*₁, which is further broken down to the 3-carbon intermediate *C*. This is then oxidized to CO_2 and water, although considerable fractions of it may be built back again by the energy of the oxidation into an unknown anabolite, *B*_{0a}, by the process of oxidative anabolism (Blackman, 1928). The question now arises as to what happens when the leaf is illuminated in an atmosphere devoid of CO_2 .

In the first place the CO_2 evolution of Cherry Laurel is reduced to about 8 per cent. of that in the dark. This phenomenon is generally accepted as due to the reassimilation of the respiratory CO_2 and not to any inhibitory effect of light on the respiratory process itself. It is, of course, possible that photosynthesis of intermediates such as *C* may take place in the light.

In the dark the concentration of sugar (*B*) most probably remains relatively constant during the protoplasmic respiration phase, giving rise, over the same period, to a steady respiration rate. The incidence of light disturbs this steady state of supply and utilization of *B* by introducing another factor, namely, increased sugar concentration by the photosynthesis of respiratory CO_2 or the intermediate *C*. The magnitude of this change in concentration is unpredictable, since nothing is known of the system controlling the hydrolysis and condensation in the $A \rightleftharpoons B$ portion of the chain.

The results of experiments have conclusively shown that the respiration of a shoot immediately after a period of illumination in both CO_2 -enriched and CO_2 -free air, as measured by the CO_2 output, is always greater than the respiration of the same shoot immediately before the illumination. This has two possible causes:

(1) The first is that the total downward flux in the reaction chain $A \rightarrow B \rightarrow C$ is increased after the illumination, assuming that, if oxidative anabolism occurs, its ratio to oxidative respiration remains a constant during the illumination and the subsequent effect. This augmented rate of downward flux slowly returns to normal in the dark.

(2) The second is that illumination causes oxidative anabolism to be wholly or partially substituted by oxidative respiration, giving rise therefore to a much-increased output of respiratory CO_2 for the same flux in the chain $A \rightarrow B \rightarrow C$. The normal values of oxidative anabolism and oxidative respiration are slowly recovered in the dark.

Let us consider the first possibility. It is highly probable that the conditions of flux existing in the chain immediately following the cessation of the light period are the same as those existing immediately preceding that cessation, i.e. the change in flux is one that occurs gradually during the illumination period under the action of light and not suddenly at the time of cessation of the illumination. This gradual accelerating effect of light on the downward flux of carbon in the reaction chain has two possible causes:

(a) An action accelerating the $B \rightarrow C \rightarrow \text{CO}_2$ portion of the chain, due to an increase in the concentration of B , as a result of the assimilation of C or CO_2 in the light. This, of course, depends on the assumption that the concentration of B limits the rate of respiration and that the reaction $A \rightarrow B$ is not affected by light.

(b) Secondly the action of light could be on the $A \rightarrow B$ portion of the chain, causing an increase in the rate of production of B from A .

Let us examine the first of these two possibilities in the light of data acquired from the previous experiments. In this case the incidence of light results in an increase in the concentration of respirable sugars. This would cause an increase in the downward flux from B and a further increase in the rate of production of B by assimilation. The net result would be an accumulation of these respirable sugars in the cell. There are two possible fates for this increased sugar content. Firstly its katabolic breakdown to C and hence to CO_2 and oxidative anabolite. The second is its anabolic condensation to A . Both these processes could go on in the dark as well as in the light. For this theory to explain the facts, however, we must assume that the condensation to A is in abeyance and a considerable amount of assimilated sugar remains as such in the cell at the cessation of the illumination period. As respiration depletes this sugar, the respiration will fall off, reaching normal when the concentration of sugar in the cell reaches the normal value for the starved leaf. If we assume that all the sugar assimilated from the respiratory products in the light accumulates in the leaf and none is condensed back to A , then the total amount of CO_2 respired in the effect should be numerically equal to the CO_2 -equivalent of sugar produced from A during the period. It has been shown, however, that the effect is many times larger (5–10 times) than could be accounted for by an accumulation of sugars from A at the normal pre-illumination rates. Thus there must be a large increase in the rate of production of B from A to account for the size of the effect.

Results of illumination in CO₂-enriched air emphasize the inadequacy of this first theory. Two tentative theories were put forward in a previous section to explain results in both CO₂-free and CO₂-enriched air, both of which involved a considerable increase of respiration (now seen to be hydrolysis of *A*) in the light. In Table II are brought together the theoretical ratios, of this respiration in the light to that in the dark, necessary to explain (on these two hypotheses) results of illumination in CO₂-enriched air.¹

TABLE II

Exp.	Starvation time (hours).	Calculated ratios of respiration rate in light to that in dark		Observed ratios R/R_0 .
		Hypothesis I.	Hypothesis II.	
III	45	2.89	8.80	1.63
	114	6.21	18.24	2.16
	189	5.84	18.40	2.14
	258	5.60	21.20	2.14
	330	5.39	26.7	2.07
	402	5.01	34.1	1.78
	474	5.60	35.9	2.08
IV	90	11.62	—	—
	162	3.07	27.4	1.61
	234	5.98	50.7	2.13
	306	—	—	1.63
	381	7.31	71.1	2.02
	474	6.34	—	1.98
	456	10.08	74.0	2.28
V	40½	8.20	321.0	1.39
	138	15.84	72.0	1.75
	208½	8.45	106.0	1.71
	280½	12.98	446.0	1.68
	376½	11.82	106.0	1.95
	448½	9.76	114.0	1.60
VII	21	1.62	—	1.12
	114	14.5	—	1.15
	186½	14.9	—	1.26
	259	10.78	131.8	1.43
	335	9.49	57.4	1.53
	419	7.27	53.8	1.76
	512	10.24	217.6	1.30

It is highly probable that the respiration rate in the light immediately preceding the cessation of the illumination is the same as that in the dark immediately after the cessation of the illumination, i.e. there is no great change in the respiration rate at the time of switch over from light to darkness. The ratio of this respiration to that immediately preceding the illumination has

In the case of hypothesis I the ratio is $\frac{Ec_{10}}{RT/180}$ and in hypothesis II it is this ratio divided by $(Ea_{10} - Ec_{10})/A_{10}$.

been determined from the graphs and appears in column 5. It will be seen that the ratios calculated on both hypotheses are very high and greatly exceed the observed ratios of column 5. It seems, therefore, that accumulation of sugars (*B*) as a result of photosynthesis is far from being sufficient to account for the observed effects.

We will therefore turn to the second possibility (*b*). Here its action would be merely on the speed of the gross reaction $A \rightarrow B$, by stimulating one or more components of the enzyme system controlling the reaction. Thus the incidence of light would cause a gradual activation of the enzyme system, which would persist after the illumination had ceased, and return to its inactivated state about 2 to 3 days after the illumination period. In this case we must assume that in the starved leaf the rate of supply of directly respirable sugars from reserve *A* is limiting the rate of respiration. Then increase or decrease of this hydrolysis rate would automatically increase or decrease the respiration rate. The activation could be of the enzyme itself, increasing the number of active centres or the specific reaction rate of one or more of the existing centres. It could also be of the nature of the destruction of a depressant of the enzyme system. The return of the respiration in the dark, to the normal value, would then be due to the subsequent reaccumulation of depressant.

This latter theory of enzyme activation has been subjected to mathematical investigation in an attempt to forecast the exact nature of the effect and its relationship with the several illumination conditions. At the outset of the investigation the following postulates have been laid down: (1) That the rate of respiration during the protoplasmic phase of the starvation life of the leaf on the shoot is controlled by the rate of supply of directly respirable sugars from reserves *A*. (2) That this supply is catalysed by an enzyme system (*E*), which is in a state of depression below its maximal activity, in the starved leaf, by a depressant (*D*), accumulated in the dark. This enzyme system *E* may be one or more components of the complex controlling the gross reaction $A \rightarrow B$, and is the limiting factor in the whole chain. (3) The action of light is to destroy this depressant and that the rate of destruction by light is proportional to the quantity of light energy falling on the system per unit time and to the concentration of depressant *D*. (4) The depressant itself is being continually reformed from the products of its breakdown according to the normal laws of mass action. The rate of its regeneration at any time is therefore proportional to the difference between the actual concentration and the maximal concentration in the normal leaf starving in the dark. (5) That throughout the protoplasmic phase of the starvation of the leaf the values of the concentration and maximal activity of the enzyme (*E*) and the maximal concentration of the depressant remain constant. This is improbable, but variations should be small during this phase of the starvation life.

With these postulates the following equations for conditions existing in the leaf have been constructed:

Let v = the rate of supply of directly respirable sugars from reserve *A*

Then, since the respiratory enzymes acting on this sugar substrate is not saturated, i.e. v is limiting the respiration rate R , then R will be proportional to v , i.e.

$$R = K_1 v \text{ where } K_1 \text{ is a constant.}$$

Haldane (1930) has shown that for competitive depression of the enzyme system the following equation holds:

$$v = \frac{K_2 ES}{S + C_1(1 + D/C_2)},$$

where E is the concentration of enzyme; S is the effective concentration of substrate, here the reserve being acted upon by E to produce the directly respirable substrate at a velocity v ; D is the concentration of depressant; C_1 is the dissociation constant of the enzyme-substrate complex; C_2 is the dissociation constant of the enzyme-depressant complex.

For non-competitive depression the corresponding equation is:

$$v = \frac{K_2 C_2 ES}{(S + C_1)(D + C_2)}.$$

It therefore follows from these equations that the respiration rate in the starved leaf is given by the expression:

$$R = \frac{KC_2 SE}{C_2 S + C_1 D + C_1 C_2} \quad \text{for competitive} \quad (1)$$

depression and

$$R = \frac{KC_2 SE}{(S + C_1)(D + C_2)} \quad \text{for non-competitive,} \quad (1a)$$

where

$$K = K_1 K_2.$$

From postulates 3 and 4 it follows that the equation for the rate of change of the concentration of depressant in the light is:

$$-\frac{dD}{dt} = k_1 LD - k_2(D_0 - D), \quad (2)$$

where D_0 is the maximal concentration of depressant in the normal starved leaf when $t = 0$, i.e. at the commencement of the illumination period, and k_1 and k_2 are constants.

The solution of this equation is:

$$D = \frac{D_0}{k_1 L + k_2} [k_2 + k_1 Le^{-(k_1 L + k_2)t}]. \quad (3)$$

Equations 1 and 3 can be combined. Let R_0 be the rate of respiration when the concentration of depressant is at the maximal value D_0 , i.e. the normal rate of respiration on the protoplasmic phase. Similarly let R be the rate after a time of illumination t , when the concentration of depressant has become D . On combining equations 1 and 3 it can be shown that:

$$\frac{1}{R_0} - \frac{1}{R} = \frac{C_1 D_0}{KC_2 SE} \cdot \frac{k_1 L}{k_1 L + k_2} [1 - e^{-(k_1 L + k_2)t}] \quad (4)$$

for competitive depression and

$$\frac{1}{R_0} - \frac{1}{R} = \frac{(S+C_1)D_0}{KC_2SE} \cdot \frac{K_1L}{(k_1L+k_2)} [1 - e^{-(k_1L+k_2)t}] \quad (4a)$$

for non-competitive depression.

Both these expressions contain S , the effective substrate concentration (reserve A). It is probable that this will drift, presumably downwards, over the protoplasmic phase of the respiration during which the series of experiments were carried out. By multiplying these equations by

$$R_0 = \frac{KC_2SE}{C_2S+C_1D_0+C_1C_2}$$

and

$$R_0 = \frac{KC_2SE}{(S+C_1)(D_0+C_2)}$$

respectively we obtain the equations

$$\left(\frac{1}{R_0} - \frac{1}{R}\right)R_0 = \frac{C_1D_0}{C_2S+C_1D_0+C_1C_2} \cdot \frac{k_1L}{(k_1L+k_2)} [1 - e^{-(k_1L+k_2)t}] \quad (5)$$

$$\text{and} \quad \left(\frac{1}{R_0} - \frac{1}{R}\right)R_0 = \frac{D_0}{D_0+C_2} \cdot \frac{k_1L}{(k_1L+k_2)} [1 - e^{-(k_1L+k_2)t}] \quad (5a)$$

for the two types of depression. In the latter case S has been eliminated and thus there should be no drift of the value of $\left(\frac{1}{R_0} - \frac{1}{R}\right)R_0 = \frac{R-R_0}{R}$ with starvation. In the first case S is still present in the denominator but the effect of any variation in this factor is diminished by the added constants $C_1D_0+C_1C_2$.

To apply the above theoretical deductions to the observed results, the value of $(R-R_0)/R$ has been calculated for all the illumination experiments carried out in CO_2 -free air. R_0 has been taken as the value of the respiration immediately preceding the illumination and derived from the smooth tracings of the respiration. The peak values of the smoothed effects, immediately after the cessation of the illumination, have been taken as the nearest approximation to R . The fit of these values to the above expressions have been tested in all cases where possible.

Four experimental series (VII, XIII, XV, and XVI) have been carried out, in which a large range of duration of illumination have been used, with constant intensities of illumination within the series. Each of these series of effects have been analysed by plotting $(R-R_0)/R$ against the corresponding values of t . These graphs are shown in Fig. 11, where the theoretical curves of nearest fit have been drawn as continuous lines. It will be seen that, with the exception of XVI, there is a fairly close fit of observed to calculated result. The low observed values in series XVI for $t = 715$ minutes are probably due to the fact that the respiration of the shoots at this time had already begun to rise in the senescent hump, where other factors come into the picture, and our initial postulates most probably do not hold.

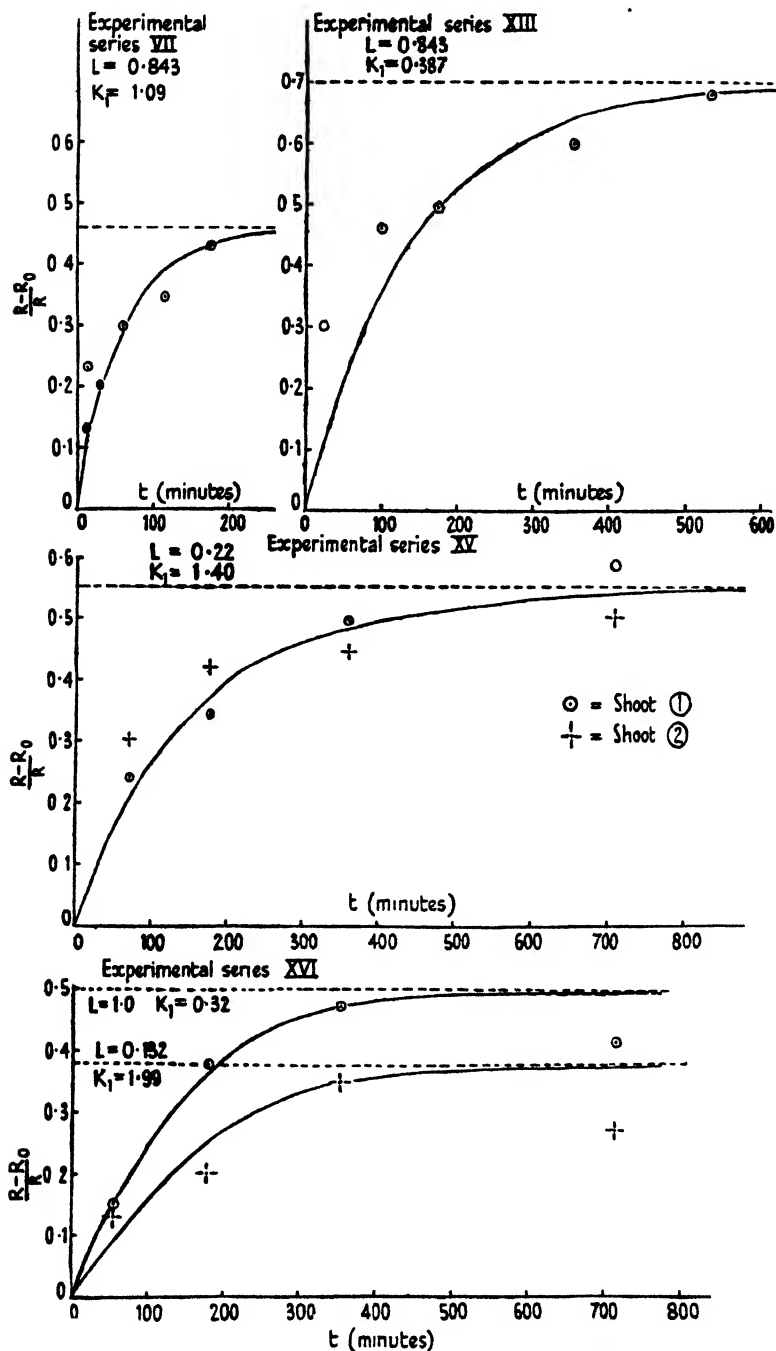


FIG. 11. Analysis on hypothesis (1) of effects of illumination in CO_2 -free air in experiment series VII, XIII, XV, and XVI. Graphs showing the fit of observed (ringed points) to theoretically deduced results (smoothed curve).

Returning to the first three graphs the deviations of observed values from calculated have been determined, in order to investigate the relationship between this deviation and the starvation age of the shoot. An analysis of these values is seen in Fig. 12. It will be seen that the observed minus the calculated values of $(R-R_0)/R$ show a marked increase towards the end of the protoplasmic phase of the starvation drift of respiration. This increase is in all cases much greater than the scatter of values about the zero axis in the early part of the protoplasmic phase. It is interesting to note that this is what

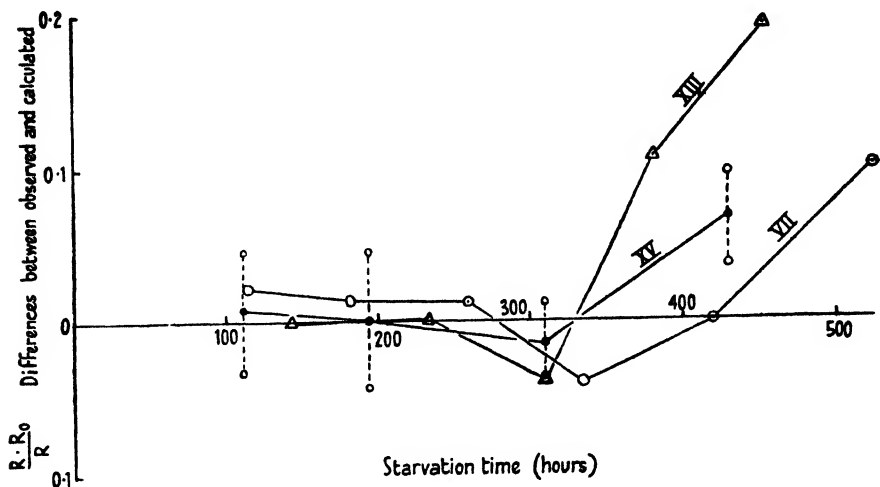


FIG. 12. Analysis on hypothesis (1) of the effects of illumination in CO_2 -free air in experiment series VII, XIII, and XV. Graphs showing the drifts with starvation of the differences between observed and calculated size of effect.

might be expected from a consideration of equation 5. Thus if, as is most probable, S decreases during the protoplasmic phase, then $(R-R_0)/R$ should show a corresponding increase irrespective of the value of L or t . These results suggest therefore that the depression is competitive, since no such increase would be expected from equation 5a.

On the same theory of depressant destruction it is possible to forecast the nature of the return of the respiration to normal after the illumination has ceased. Thus at the time of the cessation of the illumination equation 2 becomes:

$$\frac{dD}{dt} = k_2(D_0 - D). \quad (6)$$

It can be shown that if R_T is the respiration intensity at any time T after the cessation of the illumination, then:

$$\frac{R_T - R_0}{R_T} = \frac{R - R_0}{R} \cdot e^{-k_2 T} \quad (7)$$

for both types of depression. Thus the respiration of the effect should fall

away smoothly with time according to the above equation. Actually only approximately half the effects obtained (57 out of a total of 115) show a continuous smooth fall in the respiration to normal. Of the remaining 58

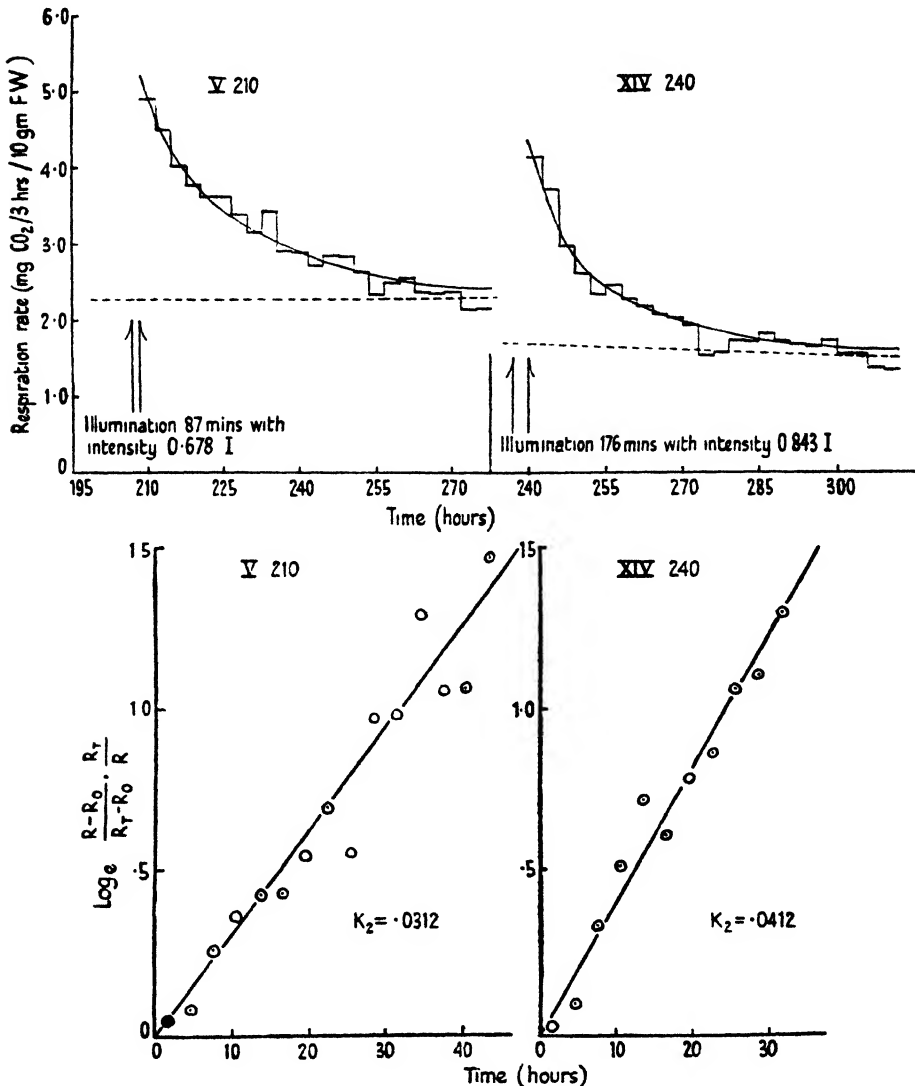


FIG. 13. Analysis on hypothesis (1) of the after-effects of illumination in CO₂-free air. Upper graphs: fit of observed respiration to theoretical recovery curve in two typical cases. Lower graphs: curves for the calculation of k_2 .

effects, 44 show a small but very definite secondary hump at about 20 hours from the cessation of the illumination (mean of 44 values = 20.3, with a standard error of 0.58). In the remaining 14 effects the interruption of the fall takes the form of a horizontal plateau lasting some 15 to 24 hours. The

cause or causes of these interruptions in the smooth fall are obscure, but they have been shown not to be due to any variations in stomatal aperture after lighting, as was at first suspected. In the case of effects showing the smooth uninterrupted fall observed results fit closely to the calculated. In the top two graphs of Fig. 13 this fit is shown in the case of two effects chosen at random from the experiments. The stepped curves are the observed respiration readings and the smooth lines the theoretical curves of nearest fit calcu-

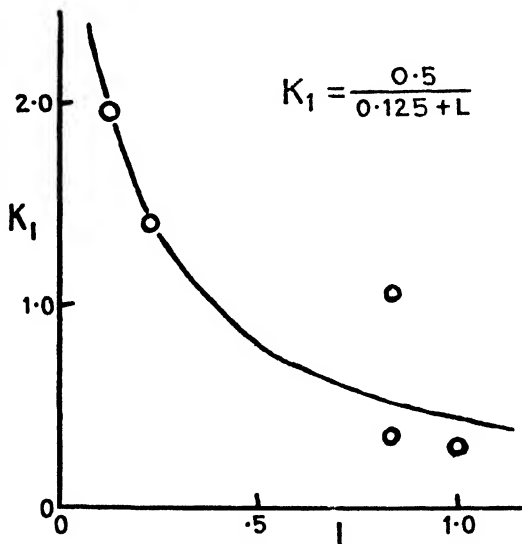


FIG. 14. Analysis on hypothesis (1) of the after-effects of illumination in CO_2 -free air. Graph showing the relationship between k_1 and the light intensity.

lated from equation 7. The dotted lines are the normal drift curves in each case.

From equation 7 we have

$$\log_e \frac{(R-R_0)R_T}{(R_T-R_0)R} = k_2 T.$$

By plotting the values of the expression on the left-hand side of this equation, calculated from the observed values of R_0 , R_T , and R , against the corresponding values of T , a straight line should be obtained. This has been done in the second two graphs of Fig. 13 for the same two effects, and a fair fit of the observed points to the straight line is seen. From the slope of this straight line k_2 can be calculated. In the case of these two effects it is 0.0312 and 0.0412. k_2 has also been calculated for all the smooth effects observed and an average value of 0.040 (standard error 0.004) was obtained.

From the theoretical curves of nearest fit of Fig. 11 the values of $(k_1L + k_2)$ and hence k_1L can be calculated for the five series at various light intensities.

On doing this, however, it was found that k_1 was not a constant, but varied with L , as shown in Fig. 14. The value $k_1 = \frac{0.5}{0.125+L}$, drawn as a smooth curve in the figure, fits the observed values fairly closely. From these values of k_1 and k_2 the theoretical curve relating $(R-R_0)/R$ to light intensity has

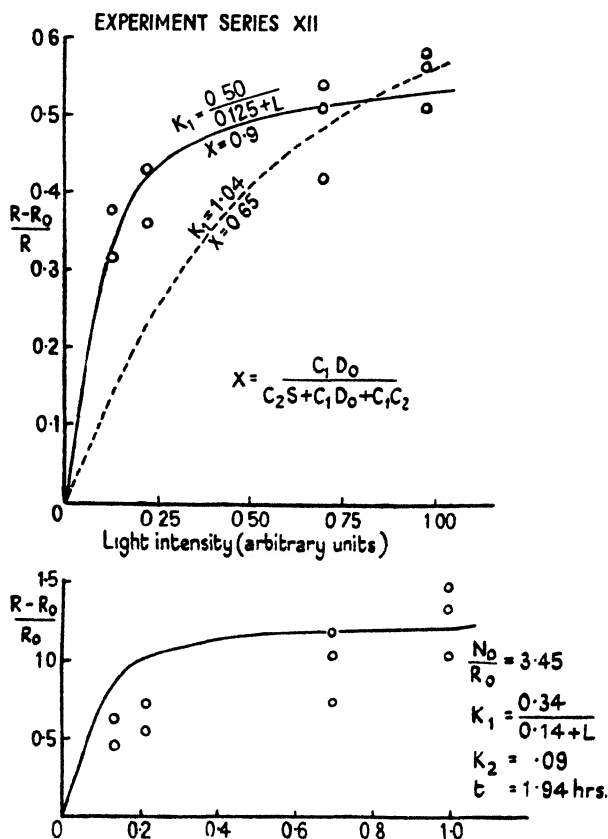


FIG. 15. Analysis of the after-effects of illumination in CO_2 -free air. The upper graph shows the fit of observed points to curves calculated from equation 5 for various light intensities. The lower graph shows the fit for a curve calculated on the theory of light inhibition of oxidative anabolism (hypothesis (2)).

been calculated from equations 5 to fit to the results of series XII. In this series the value of t varied slightly from experiment to experiment, but the average value of 1.94 hours has been taken for these calculations. A value of 0.9 for the constant $C_1 D_0 / (C_2 S + C_1 D_0 + C_1 C_2)$ gives the curve of nearest fit to the results of series XII. The observed points and theoretical curve are seen in the top curve of Fig. 15. The dotted line is the theoretical curve for a constant value of $k_1 = 1.04$, the average of the five previous series. The fit of the first curve to the points is remarkable, particularly when compared with the second curve obtained with a constant k_1 . This suggests that our initial

postulate, that depressant destruction is directly proportional to the light intensity, must be modified in the light of this subsequent analysis, and that equation 2 must now be written:

$$-\frac{dD}{dt} = \frac{k_3 L}{k_4 + L} \cdot D - k_2(D_0 - D)$$

where k_3 and k_4 are new constants, i.e. the light efficiency of depressant destruction falls off rapidly as the intensity increases.

So far this analysis has been concerned only with the results of illumination in CO₂-free air. It remains to be seen whether the results in CO₂-enriched air can be explained on the same theory. It has been seen that the effects of illumination under the latter conditions, as measured by the area of the effects curves, are always greater than the corresponding effects in CO₂-free air. The same is true for the values of $(R - R_0)/R$, which are on the average slightly greater for illuminations in CO₂-enriched air than for corresponding illuminations in CO₂-free air. From a total of 32 such illumination experiments the mean ratio of values in CO₂-enriched air to the corresponding values in CO₂-free air was 1.176 (standard error 0.037).

The foregoing equations are based on the assumption that S , the effective concentration of reserve, does not change over the period of the illumination. It is probable, however, that S may increase over this period during intense assimilation in CO₂-enriched air. If S_0 and S are these concentrations before and after the illumination period respectively, then it can be shown that, for competitive depression, the ratio of $(R - R_0)/R$, when S_0 increases to S , to the value when S_0 remains constant, is given by the expression

$$\frac{C_2(1 - S_0/S) + (D_0 - D) \cdot S_0/S}{(D_0 - D)}.$$

It is obvious that if S is greater than S_0 then this value is greater than 1, thus agreeing with the observed average ratio of 1.176. For non-competitive depression the expression is much more complicated and is not necessarily greater than 1 when S is greater than S_0 .

From the foregoing it will be seen that the results of illumination in both CO₂-free and CO₂-enriched air can be explained by the destruction in the light of a competitive depressant of the enzyme hydrolysing reserves. This was based on the assumption that if, as seems likely (see Audus, 1940), oxidative anabolism occurs as a counterpart of normal respiration in the Cherry Laurel, then its ratio to normal respiration remained constant over the period of illumination and after-effect. It is possible, however, as previously mentioned as alternative (2) (p. 188), that the effect of illumination might consist in a partial inhibition of this oxidative anabolism, the increased glycolysate thus freed being diverted and oxidized to CO₂ and water and appearing as increased respiration. If we make the assumption that the

inhibition of oxidative anabolism follows the same lines as laid down for the destruction of depressant, i.e. if

$$-\frac{dN}{dt} = k_1 LN - k_2(N_0 - N),$$

where N is the oxidative anabolism at time t and N_0 is the maximal value in the normal leaf in the dark, k_1 , k_2 , and L having the same meaning as before, then it can be shown that

$$\frac{R-R_0}{R_0} = \frac{N_0}{R_0} \cdot \frac{k_1 L}{k_1 L + k_2} [1 - e^{-(k_1 L + k_2)t}]. \quad (8)$$

Values of $(R-R_0)/R_0$ have been calculated for experiment series VII, XIII, XV, and XVI. Theoretical curves from equation 8 above show almost as good a fit to these values as the fit of observed and calculated values in Fig. 11. The values of k_1 and k_2 have also been calculated in a way similar to those of the previous analysis. In this case k_2 has an average value of 0.09 and $k_1 = 0.34/(0.14 + L)$. These values, together with those of $t = 1.94$ and $N_0/R_0 = 3.45$, have been substituted in equation 8, and $(R-R_0)/R_0$ calculated for various values of L . The resulting theoretical curve appears as a smooth line in the second graph of Fig. 15 for experiment series XII. The value of 3.45 for N_0/R_0 has been chosen to make the curve fit the observed points at what would appear to be their asymptotic value. Here the agreement of observed and calculated values is by no means as close as the curve above for the first theory, although the value of N_0/R_0 is of the order expected from direct measurement. Unpublished work by the author gave values of from 3.5 to 5.5 from measurements of relative respiration and fermentation rates in the Cherry Laurel.

It seems therefore from these analyses that the effects of illumination on the respiration is primarily plasmogeneous, although the exact nature of the effect is still rather obscure. Of the two theories put forward to explain the observed facts, that of the activation of the reserve-hydrolysing enzyme by the destruction of a depressant seems to fit the observed facts rather more closely than that of the inhibition of oxidative anabolism. It is interesting to note in this respect that the observed effects of light in bringing about opening of the guard cells of stomata is usually explained by the action of light on diastase activity, causing a shift in the starch \rightleftharpoons sugar equilibrium in the direction of increased sugar concentration. It is possible that some similar phenomenon occurs in the whole leaf in the light. It is visualized that in the light the respiration builds up with time according to equations 5 or 8, the final asymptotic value being determined by the light intensity.

It is also highly probable that the floating phase of the starvation respiration drift is nothing more than a recovery in the dark from conditions occurring immediately prior to and during gathering of the leaves. Of these conditions illumination is probably the most important, although traumatic stimulation has also been shown to play a part (Audus, 1935).

I wish to express my sincere appreciation of the stimulating interest and help afforded me by the late Dr. F. F. Blackman during the whole course of these investigations.

SUMMARY

The problem of the effect of light on the respiration of green plants has been studied in the Cherry Laurel by exposing cut shoots to various intensities and durations of illumination, and following their subsequent respiration in the dark. It has been found that this illumination, whether carried out in an atmosphere completely devoid of CO_2 , or in one containing concentrations of CO_2 of the order of 5 per cent., produced a marked stimulation of the subsequent respiration in the dark. The stimulation effect showed a maximum in the first 3 hours after the cessation of the light period, thence falling away either smoothly, or after the formation of a secondary smaller maximum at about 20 hours, to reach normal after a period of about 2 to 3 days.

Two estimates of the size of the effect have been used. The first was the total CO_2 , measured as excess above the normal drift of the respiration. The second was the height of the peak of the initial respiration maximum above the normal drift. In the case of illuminations in CO_2 -free air the first measure showed close correlations with the time of duration of the illumination and the pitch of the normal drift respiration at the time of the illumination. Again, the effects of illumination in CO_2 -enriched air were, as a general rule, larger than the effects of a corresponding illumination in CO_2 -free air, although the difference in extra CO_2 between them was never greater than a third the amount of CO_2 absorbed by the shoots in CO_2 -enriched air. Careful examination of the results show that they cannot be explained solely by an effect of an increase in the assimilates in the leaves after illumination.

Results indicate that in the light respiration builds up with time to an asymptotic value determined by the light intensity. It has been shown that the results can be plausibly explained by the hypothesis that the action of light on the respiration is to cause a stimulation of the rate of hydrolysis of reserves providing the immediate respiratory substrate (presumably hexose sugars), by destroying a competitive depressant of the hydrolysis enzyme system. A second hypothesis that light depresses oxidative anabolism, thus diverting more of the products of glycolysis to oxidative respiration, can also be shown to fit the observed data, though not so closely as the first theory.

Periods of assimilation during starvation also result in a slight raising of the starvation level of the respiration drift above that of a shoot similarly illuminated but in CO_2 -free air. This is presumably due, directly or indirectly, to the raising of the relative level of carbohydrate reserves.

Measurements have also been made of the CO_2 evolution of shoots during illumination in CO_2 -free air. The rate is reduced on the average to 0.08 of its value in the dark. There seems to be no obvious effect of light intensity on this ratio.

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Experimental and Analytical Studies of Pteridophytes

X. The Size-Structure Correlation in the Filicinean Vascular System

BY

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With eighteen Figures in the Text

INTRODUCTION

FROM a comprehensive study of pteridophytes Bower (1921, 1923, 1924, 1925, 1930) concluded that the progressive elaboration in the form of the stele during the individual development may properly be regarded as a consequence of increase in size. In announcing this generalization he emphasized the importance of a high ratio of surface to bulk being maintained in those tissues which depend for their functional success upon surfaces of transit. For, in terms of Galileo's Principle of Similitude, in any enlarging object of constant shape whereas the surface increases as the square of the linear dimensions, the bulk increases as the cube. Subsequently Bower introduced the conception of the 'size factor' by which he meant 'that influence which tends to secure by modification of form a due levelling up of the proportion of surface to bulk as the size increases. . . . It may be described as a Morphoplastic Factor. The evidence that it does act in moulding tissues is widespread, and its incidence is insistent and unavoidable.'

Although the fact that increase in size and increase in stelar complexity go hand in hand had been demonstrated on a wide basis for the shoots, leaves, and roots of pteridophytes and other vascular plants (Bower, 1930; Bouillienne, 1928; Wardlaw, 1924, 1925, 1928), the underlying mechanism was not apparent. It is difficult, for example, to explain how a protostele comes to assume a progressively more complicated outline with increase in size: nevertheless, that it does so become and that certain physiological and structural advantages may accrue may be duly recognized and emphasized.

In Bower's original statement it was implied that these advantages in some way influence the structural elaboration which attends increase in size. Moreover, it is clear that he not only regarded size as a causal factor but one which may eventually be more clearly defined by the study of differentiation during development. Such conceptions present serious difficulties. Since the term 'factor' as used in plant physiology connotes some condition which affects the rate of a process (or more generally, something which *does* something) its use by Bower in the context under consideration is inappropriate. But we owe it to him that the existence of the size-structure correlation may

now be accepted as a fact. The supporting data are set out in detail in his book *Size and Form in Plants* (1930). The mechanism by which this relationship is brought about still awaits investigation.

The arguments employed in the earlier studies by Bower and Wardlaw related almost exclusively to the functional efficiency of tissues and organs in the fully differentiated condition. As growth and differentiation are necessarily antecedent to the functional activities of the mature tissue systems, and as the distinctive configuration of the vascular system becomes apparent in the growing region below the apical meristem (Wardlaw, 1945), it is there that the factors responsible for the size-structure correlation must be studied. A comparable point of view has been expressed by Thoday (1939): he points out that tissue patterns may be determined at a very early stage of development and that the causal aspect of development may prove to be of greater significance than any consideration of the functioning of the mature organ. 'The convolutions and other changes of form which the vascular tissues undergo have functional consequences; but they also need investigation as the outcome of developmental processes taking place according to definite laws which causal analysis should reveal.' Although the structural changes which accompany changes in size may be held to be functionally advantageous, as no doubt they often are, nevertheless such advantages cannot be directly involved during the formative stage. As Thoday (1939) has said: 'It is developmental harmony which is so important, of which functional efficiency of adult organs is but a part.'

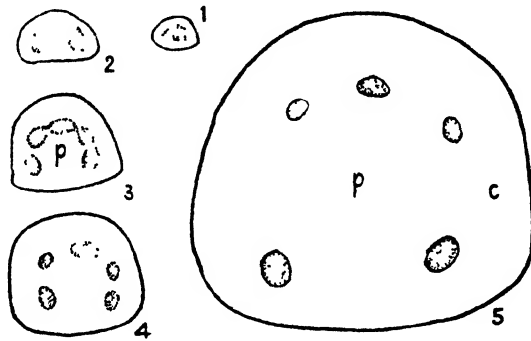
The size-structure correlation may now be accepted as a fact. The basic question is how this relationship is established. In the present paper an attempt is made to throw new light on this problem.

MEDULLATION AND THE SIZE-STRUCTURE CORRELATION

Stages in the elaboration of the filicinean vascular system include (i) the solid protostele; (ii) the medullated protostele; (iii) the solenostele; (iv) the dictyostele; (v) the perforated dictyostele; (vi) polycycly; and for the leaf, the single, binary, or multiple leaf-trace, the elaboration of the leaf-trace in general accompanying the elaboration of the shoot stele. In the individual development the progressive elaboration of the vascular system is typically associated with an increase in the size of the pith in both petiole and shoot. If it can be shown that the enlargement of the pith is causally related to changes in the form of the vascular tract, it follows that a study of the factors relating to the parenchymatous development of the pith is of fundamental importance.

Preliminary data set out in an earlier paper (Wardlaw, 1945) may be further considered here. In very young leaf primordia of *Dryopteris aristata* the incipient vascular strand or leaf-trace, as seen in transverse section, consists of a crescentic mass of small-celled tissue. As the primordium enlarges the crescentic mass also enlarges, its curvature becoming more marked. A rapidly growing parenchymatous pith now occupies the adaxial, concave side

of the stele. As the growth of the incipient vascular tissue does not keep pace with the growth of the parenchymatous pith, stresses are set up in the crescentic mass at various points and on further growth it becomes subdivided into five to seven separate strands or meristele (Figs. 1-5). This phase of growth is characterized by the parenchymatous development of groups of cells of the incipient vascular tissue in the afore-mentioned regions of subdivision. This transformation of incipient vascular tissue into parenchyma in regions under tensile stress is clearly a matter of considerable importance and one which merits further investigation. The regular distribution of the several meristele which collectively constitute the leaf-trace may



FIGS. 1-5. *Dryopteris aristata*. Transverse sections of leaf primordia at different stages of development, each being taken at the level where the adaxial surface was about to join the shoot. Incipient vascular tissue is stippled; p, pith; c, cortex. ($\times 24$.)

be referred to the tendency towards equilibrium which is characteristic of all dynamic systems. The disruption of the vascular system in the leafless, stoloniferous tubers of *Nephrolepis cordifolia* (Sahni, 1916; Wardlaw, 1945) may be explained in the same general terms (Fig. 6). In the petiole of ferns such as *Osmunda regalis*, in contrast to the condition seen in *Dryopteris*, the growth of vascular tissue keeps pace with the enlargement of the pith and no disruption takes place. In both *Dryopteris* and *Nephrolepis* the illustrations show that the enlargement of the organ is mainly due to the great increase in the parenchymatous tissues of pith and cortex and only to a minor extent to the enlargement of the vascular system. These data give an indication of factors which are at work during the process of growth and which may be included among those which bring about the size-structure relationship.

The effect of leaf development on the vascular system of the shoot may now be considered. In *Dryopteris* (Figs. 7-10), the incipient vascular tissue below the apical meristem, as seen in transverse section, is disposed in the form of a ring with pith parenchyma developing internally and cortical parenchyma externally (Wardlaw, 1945). As Priestley (1928) has pointed out, incipient vascular tissue in this position is under pressure due to the rapid expansion of parenchymatous tissue on either side of it. If no other factors intervened to disturb the equilibrium during growth, the plastic vascular ring

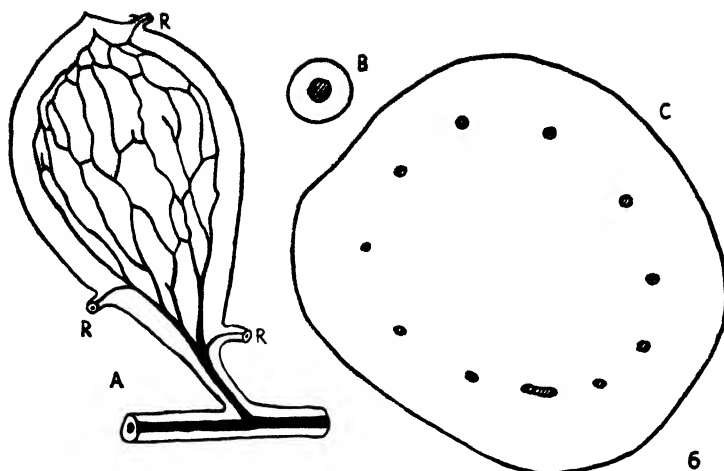
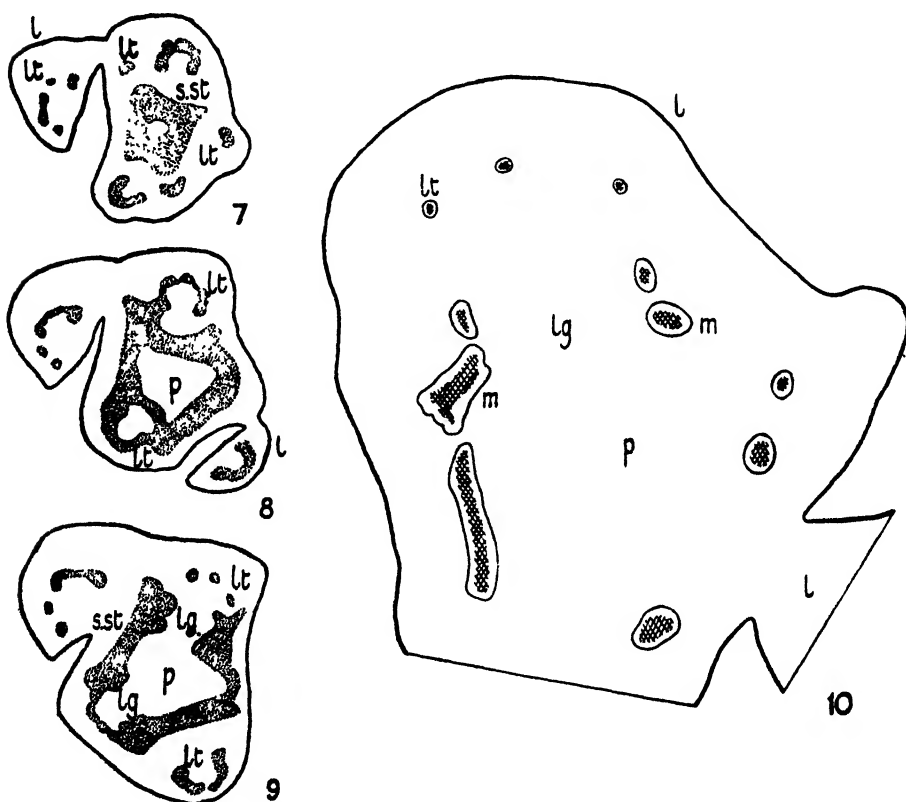


FIG. 6. *Nephrolepis cordifolia* (after Sahn). A, stolon bearing a tuber in which the proto-stele enlarges and becomes dictyostelic and then contracts again at the apex; B, C, transverse sections of protostelic stolon and tuber respectively. ($\times 5$.)



FIGS. 7-10. *Dryopteris aristata*. Transverse sections of the apex of a small shoot in basipetal sequence. Incipient vascular tissue is stippled; unligified xylem is cross-hatched; l, leaf; lt, leaf-trace; s.st., shoot stele; lg, leaf-gap; p, pith; m, meristele. Figs. 7, 8, just below the apical meristem; Fig. 9, first appearance of leaf-gaps; Fig. 10, meristeles separating. ($\times 18$.)

would increase in diameter and might become thin, but no disruption would take place except in such extreme cases of enlargement as that of *Nephrolepis* cited above. The development of leaf primordia does in fact disturb the equilibrium within the shoot. The extensive tangential expansion of the leaf base, particularly of its pith, subjects localized regions of the incipient vascular cylinder of the shoot to tensile stress. As in the leaf primordium, this is attended by the transformation of some of the incipient vascular tissue into parenchyma, the subsequent growth and development of this parenchyma giving rise to the leaf-gap. The continuity of the vascular cylinder or solenostele is thus typically interrupted in the regions of insertion of the leaf-traces. Where the leaf-gaps overlap the vascular system becomes a meshwork or dictyostele and in cross-section is seen to consist of a ring of more or less widely spaced meristeles. In experiments with *Dryopteris*, in which all the young leaf primordia were systematically excised, it has been shown that leaf-gaps are not formed, the vascular system being typically solenostelic (Wardlaw, 1944a). An alternative suggestion, that the development of parenchymatous leaf-gaps is referable primarily to the deflection of metabolites from the shoot into the developing leaf primordia, appears to the writer to be less feasible. Here the underlying idea is that the incipient vascular tissue of the shoot immediately above the level of a leaf insertion is deprived of nutrients and a parenchymatous development ensues. Such an explanation would not, however, account for the disruption of the initially unbroken leaf-trace of *Dryopteris*, for the vascular changes in the leafless tubers of *Nephrolepis*, or for the condition in those shoots where the leaf-gap extends below as well as above the insertion of the leaf-trace, e.g. *Matteuccia struthiopteris*.

In some instances the development of vascular tissue keeps pace with the expansion of the pith as in the large solenostelic rhizome of *Saccoloma*. In other instances a solenostele may be present during the earlier stages of development of the individual, but sooner or later the further expansion of the pith leads to a disruption of the vascular cylinder as in *Pteridium* or *Stenochlaena*. In the resulting *perforated dictyostele* not all of the parenchymatous gaps are caused by the insertion of the leaf-traces. These facts can be ascertained not only by tracing the development of the stele from the base of the plant upwards but also by observing the structural development in the apical region of the adult shoot. The relative developments of cortical, leaf-gap, and medullary parenchyma and of vascular tissue are thus seen to be of fundamental importance in determining the tissue-pattern at different levels in the shoot.

Taken collectively, the data set out above show that at least some of the factors responsible for the size-structure relationship can be indicated by appropriate investigation of the shoot apex.

INCEPTION OF MEDULLATION

In the development of leptosporangiate ferns a stage is reached when the enlarging protostele at the base of the shoot becomes medullated. That is to

say, the centre of the stele is occupied by normal parenchyma instead of by a solid core of tracheides or of tracheides and small-celled parenchyma. The more centrally placed incipient vascular tissue has, in fact, undergone what the older morphologists described as 'a change of destination'. On proceeding upwards in the shoot the stele is seen to enlarge, the pith becoming increasingly conspicuous. Eventually, with the appearance of internal phloem and endodermis, the typical solenostelic condition is established. In the region of insertion of each leaf-trace the parenchymous tissue is continuous from the cortex into the pith.

The inception of medullation is of critical importance in the subsequent development of the vascular system. At present very little is known of the factors which are causally related to this parenchymatous development in the centre of the xylem. Bower has pointed out that the transition from the solid to the medullated condition is typically associated with increase in size. He has also suggested that phyletically the origin of the pith is to be sought in a loss of conductive function in the central core of tracheides. His argument is that with increase in size and with water being mainly withdrawn from the outer tracheides there would tend to be a stagnation of water in the more central ones. Hence their conductive function would eventually disappear and their place would be taken by a water-storing parenchymatous pith. But, as has already been indicated, whatever the merits of such arguments may be, the development of each individual solenostele has to be accounted for in terms of the process of growth.

Medullation normally coincides with the rapid growth in thickness of the shoot as a whole. It has already been seen that incipient vascular tissue which is subjected to tensile stress tends to develop as parenchyma. If, therefore, the outer tissues of an enlarging protostele are growing more rapidly, particularly in the tangential direction, than those at the centre, the latter will be subjected to tensile stress and a condition which makes for the development of parenchyma will be established. This mechanical hypothesis, if proven, would, however, afford only a partial explanation of what is undoubtedly a complicated situation. As the facts of observation show, the enlargement of the pith in the individual development may be very extensive. It may also be noted that the pith of erect exposed leafy shoots is much larger than that of underground rhizomatous shoots, as in *Dryopteris aristata*.

Elsewhere (Wardlaw, 1947) it has been shown that in certain situations a solenostele may also result from the medullation of an incipient meristele. Solenostely should therefore not simply be regarded as an expression of the specific reaction system at a certain stage in the individual development but should be related to the interaction of factors during growth and development.

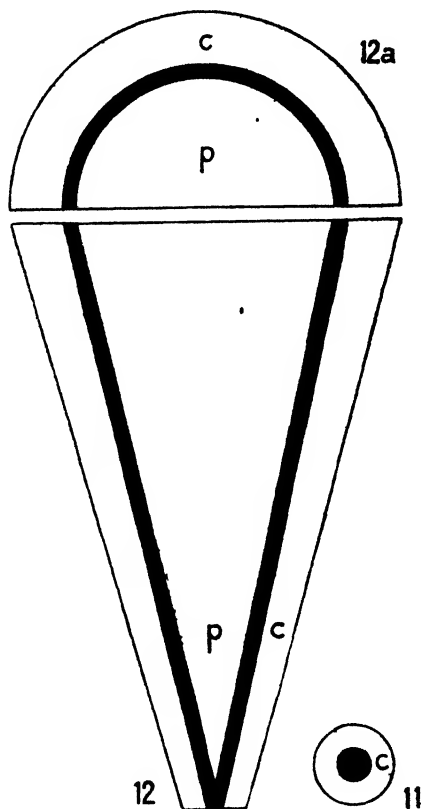
CORTEX, STELE, AND PITH

In an enlarging shoot every unit of increase in the radius requires 6.28 units of increase at the circumference if the latter is not to become disrupted. The result of this relationship is evident in shoots where the total amount of

vascular tissue is small but where the pith develops to large size, e.g. the tubers of *Nephrolepis cordifolia*. It is probable that many other aspects of growth and differentiation are also influenced to some extent by this geometrical relationship.

The development of the cortex, stele, and pith of a solenostelic shoot may now be briefly considered. Mathematically, the area relationships of concentric circular bands are involved. Fig. 11 shows the solid protostele at the base of the shoot just prior to the appearance of the pith. As the hypothetical shoot, Fig. 12, enlarges it is assumed that the thickness, i.e. the radial dimension, of the vascular tissue remains constant and that the cross-sectional areas of both pith and cortex are increased by equal amounts at each successive level. The large cross-section shown in Fig. 12a indicates the result. The pattern thus produced closely resembles that found in some large solenostelic rhizomes, e.g. *Cibotium Barometz*. The ratio 'radius' cortex: radius pith, which is initially high, falls rapidly and asymptotically and eventually reaches an approximately steady value of 0.41. The ratio radius shoot: radius stele also falls as the system enlarges. The notable feature is the obvious increase in the diameter of the pith and the relatively small increase in the width of the cortex. The relationship between the cross-sectional areas of cortex and pith in the upper region of the shoot is one which is not

evident until the actual measurements are before us. The reason is that whereas a given increment of area may produce a very considerable increase in the radius of the pith, the same increment, distributed over the outermost cylinder, produces only a small increase in the thickness ('radius') of the cortex. Measurements of the cross-sectional areas of the pith and cortex which have been made for a number of fern shoots (Wardlaw, 1945) show that they approximate closely to the relationship indicated above. It is not suggested, however, that the approximately equal enlargement of cortex and pith during growth is due to the diffusion of equivalent amounts of metabolites centrifugally and centripetally from the vascular cylinder.



FIGS. 11, 12. Diagram of stelar development in a fern. Fig. 11, solid protostele at base of shoot; stele, solid black; c, cortex. Fig. 12, longitudinal section of enlarging shoot; c, cortex; p, pith; stele, solid black. Fig. 12a. Adult region of shoot in transverse section. (See text.)

In the system under consideration (Fig. 12), it is assumed that the development of vascular tissue is such as to maintain a continuous layer round the pith. If, however, this development had been less, then the vascular ring must either have diminished in width or have become disrupted: the solenostelic rhizome of *Cibotium Barometz* affords an instance of the former, the tuber of *Nephrolepis cordifolia* of the latter.

In solenostelic and dictyostelic systems the parenchymatous gaps occur only in the regions of insertion of the leaf-traces. It seems probable that the development of the leaf-gap should be referred to the tensile stress developed in the incipient vascular cylinder both by the tangential enlargement of the leaf-base and the expansion of the medullary region of the shoot.

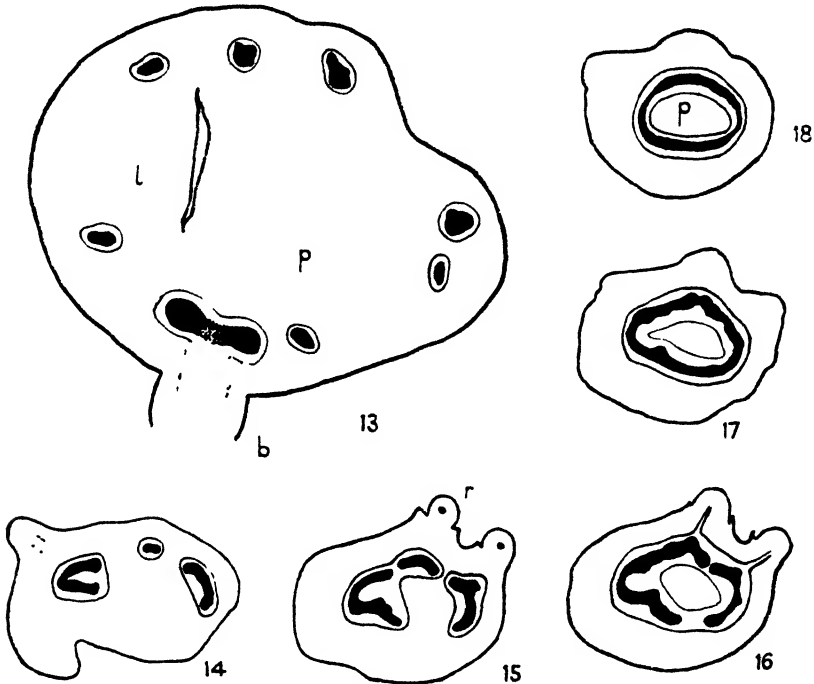
Even within a single species, e.g. *Dryopteris aristata*, solenosteles developed under different conditions may be of very different sizes. Observations of this kind proved a 'stumbling-block to Bower's original theory, and he has presented a series of drawings which show that 'the disintegration of the stele does not depend on absolute size alone'. The evidence set out here suggests that it is the different rates of growth of pith and vascular tissue that determine the configuration of the stele. Elsewhere it has been shown that the cavity in which the stele is suspended in the genus *Selaginella* can be referred to the different rates of growth of cortical and stelar tissue (Wardlaw, 1925).

POLYCYCLY, GAMOSTELY, AND REDUCTION

Enlarging shoots in which polycyclic steles are eventually developed, e.g. *Matonia pectinata*, *Paesia podophylla*, *Platyserium* spp., &c., afford unmistakable evidence of the size-structure correlation, but a detailed account of the apical region of these ferns has yet to be obtained. Further discussion of these interesting materials must therefore be deferred.

Instances are known among the ferns in which disintegrated steles (i.e. dictyosteles and perforated dictyosteles) undergo a reduction in size in relation to unfavourable conditions of growth. In such materials, on proceeding upwards in the shoot, a coalescence of the separate meristeles and the re-establishment of a solenostelic condition appear to take place. This phenomenon has been described as *gamostely*. It is now apparent that this term, though aptly descriptive of the structural changes to be seen in successive acropetal sections of the attenuated shoot, is nevertheless misleading as an interpretation of what has actually taken place. Below the apical meristem in solenostelic and dictyostelic ferns, the incipient stele, as seen in cross-section, is an unbroken ring. An account of the disintegration of this ring during development has already been given in terms of medullary enlargement in shoot and leaf-base. It follows that the so-called gamostely is not due to a fusion of parts initially separate, but is explained by the fact that the pith, being feebly developed, does not cause a disruption of the ring of incipient vascular tissue. The experimental elimination of leaf primordia and therefore of leaf-gaps in dictyostelic ferns (Wardlaw, 1944a), typically yields what would formerly have been described as evidence of gamostely. So, too, as the distal

region of the tuber is approached in *Nephrolepis cordifolia* there appears to be gamostely; but this condition is now seen to be no more than a consequence of the diminishing radius of the pith, the small incipient vascular ring being no longer subject to distension and disruption. All the instances of gamostely so far recorded, e.g. *Deparia Moorei* as described by Thompson (1915), are characterized by a marked reduction in the diameter of the pith. A typical



FIGS. 13-18. *Onoclea sensibilis*. Successive acropetal transverse sections of an attenuated rhizome showing apparent gamostely. Fig. 13, normal dictyostelic region of shoot. Figs. 14-16, attenuated region of shoot, showing 'fusion' of the separate meristeles. Figs. 17-18, the solenostelic condition eventually established. Xylem, solid black; *l*, leaf, with binary leaf-trace; *p*, pith; *b*, bud; *r*, root. ($\times 8$.)

example is illustrated in Figs. 13-18 for *Onoclea sensibilis* (Wardlaw, 1944a). In stout dictyostelic rhizomes which have been disturbed or subjected to unfavourable conditions the further growth of the shoot may also be marked by a wide spacing of the hitherto closely set leaves. In such shoots the vascular system may return to an approximately solenostelic condition. An example of fluctuation in size in a solenostelic system has been described by Thompson (1919): in *Platyzoma microphyllum* he observed that the solenostelic rhizome sometimes underwent reduction to a medullated protostelic condition with concomitant reduction in the diameter of the pith. The writer (Wardlaw, 1944a, 1945a, 1946, 1946b) has given detailed accounts of the anatomy of attenuated shoots of *Onoclea sensibilis*, *Osmunda regalis*, *Todea barbara*, and *Angiopteris evecta*, produced under different experimental

conditions. In each instance the elaborate stele in the stout region of the shoot was reduced through successive stages to a protostele, which compared closely in size with the protostele at the base of the young sporophyte. The progressive decrease in stelar elaboration was accompanied by a reduction in the size of the pith.

The Root Stele

In a survey of root steles of pteridophytes and flowering plants the writer (Wardlaw, 1928), following earlier investigations by de Bary (1884), showed that there is a well-marked correlation between the size of the stele and its structural complexity. For example, in the Marattiaceae, whereas small root-lets show monarch, diarch, or triarch structure, stout roots may have as many as twenty radiating xylem plates; while roots of intermediate size exhibit an intermediate degree of complexity. This progressive elaboration which accompanies increase in size was regarded by the writer as a tendency for a due proportion of surface to bulk to be maintained between the dead tracheides and the associated living tissue. Comparable structural developments are typical of the shoot steles of *Psilotum* and *Lycopodium* and have been discussed in the same general terms (Wardlaw, 1923). This type of explanation is open to the criticisms that have already been urged. That a large surface of interchange between tracheides and living parenchyma is consistently maintained cannot be denied by anyone who has examined small and large roots of primary structure (see also Bouillienne, 1928) but it now seems improbable that such functional relationships are causally involved in the actual process of growth and differentiation. Following the conception of Goebel (1922) on the repetitive occurrence of pattern during development, and the constancy of scale of such units of pattern at the time of inception, Thoday (1939) has indicated how the increasing complexity of structure in roots of progressively larger size could be accounted for. The strands or plates of xylem and phloem, which alternate regularly and radiate out from the central pith, may be regarded as the units of a repetitive pattern. The pattern is determined in the growing region just behind the apical meristem. If, now, the unit of pattern at its inception varies in dimensions only within narrow limits, then the number of repetitions will be determined by the space available for development, i.e. by the size of the incipient root stele. While such a conception contributes materially to our knowledge of the progressive elaboration of the root stele with increase in size, the factors which are causally related to the differentiation of the unit of pattern still await elucidation.

DISCUSSION AND CONCLUSIONS

A re-examination of Bower's views as to how the size-structure relationship is established in the vascular system in ferns has shown that new light is shed on this problem by a consideration of certain factors which are at work during the process of growth at the apex. Among surfaces of interchange which

might become 'limiting' with increase in size, Bower (1921) called attention to the endodermis and Wardlaw (1923) to the living parenchyma contiguous with the dead tracheidal conducting tissue, the data presented and the arguments adduced tending to show that the changing outlines to be observed during stelar enlargements were such as to make for a maintenance of these surfaces of interchange. But it was not then evident how these functionally useful changes were brought about. The view which has emerged from the present study is that the maintenance of stelar surfaces in enlarging shoots is a direct and necessary consequence of the action of certain factors in the growing region. In particular, the importance of certain geometric, mechanical, and metabolic aspects has been indicated.

The relative development of cortical, vascular, and medullary tissue as contributing to the changing cross-sectional pattern at different levels in the enlarging shoot has been examined with interesting results. Some of the changes in the form of the developing vascular cylinder, for example, can be attributed to the stresses developed in it by the more rapid growth of the adjacent parenchymatous tissues of the leaf-base and central medulla. The action of other factors which may be important in determining the morphology of the vascular system can also be indicated in a general way. Fundamentally the solution of the problem of the establishment of the size-structure relationship calls for an investigation of the factors which relate to the initial differentiation, position, nutrition, and rates of growth of the several tissue systems at the shoot apex. During the development of the individual fern plant the apical meristem undergoes a notable increase in size (Wardlaw, 1944). The diameter of the adult shoot stands in a direct relationship to the size of the apical meristem, i.e. other things being equal, the larger the meristem, the larger will be the adult shoot which develops from it. The stele shows a similar relationship, the initial differentiation of vascular tissue in pteridophytes being held to be directly related to the metabolic activity of the apical meristem (Wardlaw, 1944).

The size of the apical meristem and its metabolic activity, which are seen to be of special interest in the present connexion, may at any particular time be referred to (i) the nature of the specific reaction system (or specific hereditary substance), (ii) the environmental conditions, and (iii) the stage of development reached. Whereas the initial differentiation of the several tissue systems relates to a phase which is predominantly one of protein metabolism, the subsequent enlargement and maturation of the tissues, particularly of the parenchyma, constitute a phase which is predominantly one of carbohydrate metabolism. Both aspects of metabolism, in so far as they can be separated, are ultimately an expression of the interaction of genetical and environmental factors. Metabolic processes, spatial relationships, mechanical and other factors, may be held to determine the distinctive form and structure by which the individual species is recognized. These considerations raise an interesting problem for, as Bower (1924) has pointed out, to the extent that the development of specific structures can be correlated with changes in size, so do they

'lose grade as a basis for phylesis'. The results of causal and phyletic studies may in fact be at variance. Further investigations along the lines indicated here, by increasing our knowledge of the part played by extrinsic and intrinsic factors during development, may eventually make it possible to harmonize causal and phyletic views.

In the present paper criticisms have been made of the theory of the 'size factor' as originally propounded and elaborated by Bower. But criticism of detail should not be allowed to obscure the value of the generalization nor the interest which it has stimulated in problems of causality. The data which Bower presented and his insistence on the importance of accumulating relevant facts will not be neglected by any serious inquirer. It is proper that attention should be directed to a point of view expressed by him many years after the initial publication: 'What is needed in this whole organographic problem of Size is an extended investigation of the organic apex . . . the centre and focus of the whole problem lies not in the details of the adult state, though these may suggest what are the functional requirements for biological success; but in that imperfectly known and still problematical region of initiative, the Growing Point. It is here, rather than in the matured parts, that the key to causality should be sought, and the Size-relation tested by comparative measurement' (Bower, 1937).

In the present study, factors which may influence the initial differentiation and gross morphology of the vascular system have been chiefly considered. But within the vascular system, or any part of it such as a root stele or meristele, there is the further differentiation of the incipient tissue into phloem, xylem, conjunctive parenchyma, &c. There, also, as a result of the action of factors concerning which very little exact information is available, definite structural configurations or patterns are produced; and these patterns, as root studies have shown, may afford evidence of the size-structure correlation. The question has often been asked why cells, which initially are fundamentally alike, should show such marked differences during their subsequent development. Moreover, it is not merely the range in cellular differentiation that is such a remarkable phenomenon, but the fact that this differentiation takes place in an orderly manner so that a characteristic pattern is typically produced, as in a root stele or protostele. Two adjacent meristematic cells may develop into quite different kinds of mature cells, e.g. tracheide and xylem parenchyma; and this kind of differentiation takes place throughout the whole tissue-mass of the stele. Sinnott (1942) has ascribed this phenomenon to the fact that each cell 'differentiates in a way that is proper for the position in which it occurs', thus supporting the view announced by Vöchting in 1878 in the following terms: 'The particular function (character) assumed by a cell is determined primarily by the morphological position which it occupies in the living unit.'

The spatial position of a cell in a growing organ, then, determines how it shall become differentiated; the differentiation of adjacent cells yields pattern or structural configuration of a characteristic and relatively constant kind;

and pattern itself is a manifestation of the orderly development or organization which is characteristic of living things. Sinnott has raised the question as to why the mere position of a cell in a system of cells should determine the course of its subsequent differentiation. The origin of pattern, which results from differentiation, he describes as 'the most difficult task in biology. . . . There is yet no solution nor even a very active expectation of one. The very terms "pattern" and "field" are suspect to many as implying agencies too mystical or immaterial for orthodox biology.' The truth of this view must be recognized. Any explanation of the underlying causes of differentiation must necessarily be tentative and will usually represent an over-simplification of a difficult and complex situation. Nevertheless, as it appears to the writer, the point of view developed here, arising from a reconsideration of Bower's views, does give some indication of the factors—mechanical and metabolic—involved in differentiation. Thus a cell, or group of cells, occupying a particular position in the growing region of a shoot, leaf, or root, are subject to certain stresses. These stresses, which may be either compressive or tensile, will vary in different regions of the developing organ and may exercise an effect on the interchange of metabolic substances between cells. Moreover, the distribution of nutrients, and of metabolic substances generally, is not uniform over the whole growing region. Acropetal and basipetal diffusion gradients are involved, while centripetal and centrifugal gradients of gaseous diffusion may also be important. Again, for geometric reasons, some tissues have a greater degree of freedom for enlargement than others. Already, therefore, several sets of factors have been indicated which, acting together, will tend to produce an orderly differential development of tissues. In the course of this differentiation, tissues with different rates of growth may be produced and this will lead to further structural elaborations. In the case of the stele it has been seen that a progressive elaboration accompanies increase in size. The remarkable fact is that the changes in structure which accompany changes in size are such as to be probably of functional importance. The mechanism by which this biological adaptation is brought about is complex and one of which we have very little knowledge. Many interrelated factors are undoubtedly involved: some of those to which attention has been directed here may play some part in determining the change of structure with size.

SUMMARY

Bower's theory of the 'size factor' has been re-examined and criticized on the grounds that it attempts to explain the origin of the size-structure relationship in the filicinean vascular system in terms of the functioning of the various adult tissues concerned. As a result of his work the size-structure correlation may be accepted as a fact: what is still required is an adequate account of the mechanism by which the relationship is brought about. The factors responsible for the differentiation of the vascular system, and therefore for bringing about the size-structure relationship during the development of the individual,

are at work in the apical growing region and must be studied there. Many interrelated factors are involved; among factors of particular interest are those which relate to the size of the apical meristem, the initial differentiation and position of the vascular tissue, and the rates of growth of the cortical, vascular, and medullary tissues.

Data relating to the initial differentiation of vascular tissue, the medullary development in leaf-base and shoot, and the different rates of growth in adjacent tissues are given, and mechanical and metabolic factors which may be involved in bringing about the size-structure relationship are discussed. Consideration is also given to the differentiation of the several tissues within the stele.

The conclusion is reached that the size-structure correlation observed in the mature vascular system of ferns is due to the action of many interrelated factors of which little is known. Factors which may play some part in determining the change in structure of the vascular system with change in size include (i) the position occupied by the incipient vascular tissue, (ii) stresses which arise in it in relation to the more rapid growth of adjacent tissues, and (iii) metabolic factors.

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Wilt of Cacao Fruits (*Theobroma Cacao*)

IV. Seasonal Variation in the Carbohydrate Reserves of the Bark and Wood of the Cacao Tree

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INTRODUCTION

WILTING of immature fruits of the cacao tree has been demonstrated to be due to competition for nutrients between developing fruits; in young trees competition from the foliage is also an important factor (Humphries, 1943, 1943*a*, 1944). It became apparent that any attempt to elucidate the problem further would involve a consideration of the carbohydrate and mineral nutrition of the cacao tree. As a first step an investigation of the fluctuations in nutrient reserves of the cacao tree in response to environmental changes, and to the development of the crop was undertaken. It was hoped that this knowledge besides indicating the effect of the 4-5 months' dry season in Trinidad might also give some clue to the nutrients concerned in the competition. The following is a description of an experiment designed to throw further light on the relation between the nutritional status of cacao trees on a particular site and their cropping capacity. The carbohydrate data form the subject of the present communication, while a consideration of the mineral data will be deferred to a later paper.

METHODS AND PROCEDURE

A field of mature cacao trees, approximately 25 years old, situated at River Estate, Trinidad, was selected for the experiment. These trees are genetically heterogeneous, and may be regarded as representative of Trinidad plantation

¹ Now of Rothamsted Experimental Station, Harpenden, Herts.

cacao. The trees on an area of approximately 2 acres were individually surveyed and graded according to size. Three hundred trees, falling into the largest size class, were tagged with serial numbers. These constituted the experimental trees and were divided into 28 groups by drawing numbers at random. Each group consisted of 10 trees and the trees of each group were scattered over the whole area. Visits were made at approximate intervals of 4 weeks, and on each occasion 2 groups of 10 trees were sampled. The procedure for sampling an individual tree was as follows: At a distance of 1 ft. from the ground a piece of bark about 2 in. square was cut out with a sharp knife and placed in a tin with a tight-fitting lid. The bark separated readily from the wood at the cambial layer. A sample of wood at the same level was obtained by the use of a $\frac{3}{4}$ -in. auger, the turnings being collected in a tin. The boring extended as nearly as possible to the centre of the trunk. The procedure was repeated at heights of 6 ft. and 12 ft. from the base of the tree. The bark samples and the wood samples were collected in separate containers, as also were the samples from each level. Similarly, samples were collected from the remaining 9 trees of the group. The bark or wood samples at any one level from the 10 trees were bulked together. Samples were taken in a similar manner from another group of 10 trees, thus providing duplicate samples with which to obtain an estimate of the variability of the material. As each tree was visited a note was made of the number of fruits present, falling into the following categories: (1) very small, (2) small, (3) medium, (4) full, (5) ripe, (6) diseased. These data provided an estimate of the seasonal trend in fruiting condition of the trees. A note was also made of the general vegetative condition (presence or absence of a flush) of the whole experimental plot. Sampling began at 9.0 a.m. and the first sample (A) was completed by 10.30 a.m. when the second sample (B) was commenced. The samples were conveyed as quickly as possible to the laboratory where the bark was weighed. A subsample was cut into small pieces and plunged into boiling alcohol, and stored for subsequent extraction and analysis. The wood samples were found to be sufficiently finely divided to be put into boiling alcohol immediately after weighing. Subsamples of both bark and wood were dried in the oven at 105° C. for determination of water and subsequent mineral analysis. Carbohydrate determinations were made by the methods previously employed (Humphries, 1943*a*).

The chemical analyses provided 84 values for each constituent in the bark or wood (one at each level in duplicate on each of the 14 sampling occasions) and the data have been subjected to an analysis of variance subdivided according to the following scheme:

Variance due to:	Degrees of freedom.
Seasons	13
Levels	2
Interaction of seasons and levels	26
Paired samples	41
Error	42
Total	83

The data also permit of analysis of covariance between different constituents in the bark or wood and between like constituents of bark and wood.

In the following account the seasonal changes of constituents are first considered, but for convenience of reference the essential deductions from the statistical calculations have been summarized in Table I.

SEASONAL CHANGES IN CONSTITUENTS OF BARK¹ AND WOOD

(a) *Water content*

The trend of water content of the bark at the three levels expressed on a fresh-weight basis are given in Appendix Table I, and represented graphically in Fig. 1, where the averages of the A and B values at any sampling time have been plotted. The changes in water content at the three levels run practically parallel to one another and peak values occur in December, April, and August. The water content is higher at the 12-ft. level than at the 1-ft. level, with the 6-ft. values usually occupying an intermediate position. From Table I it may be seen that there is a highly significant seasonal change in the water content of the bark during the period of experiment. The difference between levels is also highly significant. The interaction between seasons and levels is not significant, indicating that the seasonal changes affect all three levels more or less to the same extent. In considering the bark data, however, it must be pointed out that the water content is apparently affected by external atmospheric conditions. It has been shown from dendrograph records (Humphries, 1943*b*) that showers of rain cause swelling of the bark of the cacao tree, and this probably indicates an increase in water content of the bark during rain. This 'hydration' effect had previously been recorded by MacDougal (e.g. MacDougal, 1938) for certain other species of trees. It may be surmised that the hydration effect is confined to the peripheral layers of the bark and affects the gross water content of the bark but slightly, so that a real cycle of water content change as indicated in Fig. 1 probably exists. The swelling of the bark during rain may possibly be partly attributed to the effect of extraneous water on mucilage contained in numerous ducts in the outer cortex of the bark (see Fig. 8).

There is a distinct seasonal trend in the water content of the wood. At all three levels the values fall from January onwards as conditions become drier (cf. rainfall graph, Fig. 1). This decline in water content is not so marked at the 1-ft. level as at the 6-ft. and 12-ft. levels, although there is a small increase in the water content of the wood at the 12-ft. level, reaching a minor peak in April. The reason for this is not clear. With the onset of the rains in July the water content of the wood increases again at all levels to the former wet-season values. Thus during the dry season in Trinidad there is a definite progressive decrease in water content of the wood which might have a harmful effect if the drought is unduly prolonged. The seasonal variation in water content of the wood is very highly significant, as also is the difference between

¹ The term 'bark' in this paper refers to all tissues external to the cambium.

levels (see Table I). There is no interaction between seasons and levels. It will be noticed that the water content of the wood is lowest at the 12-ft. level, i.e. the gradients of water content are directly opposed in bark and wood.

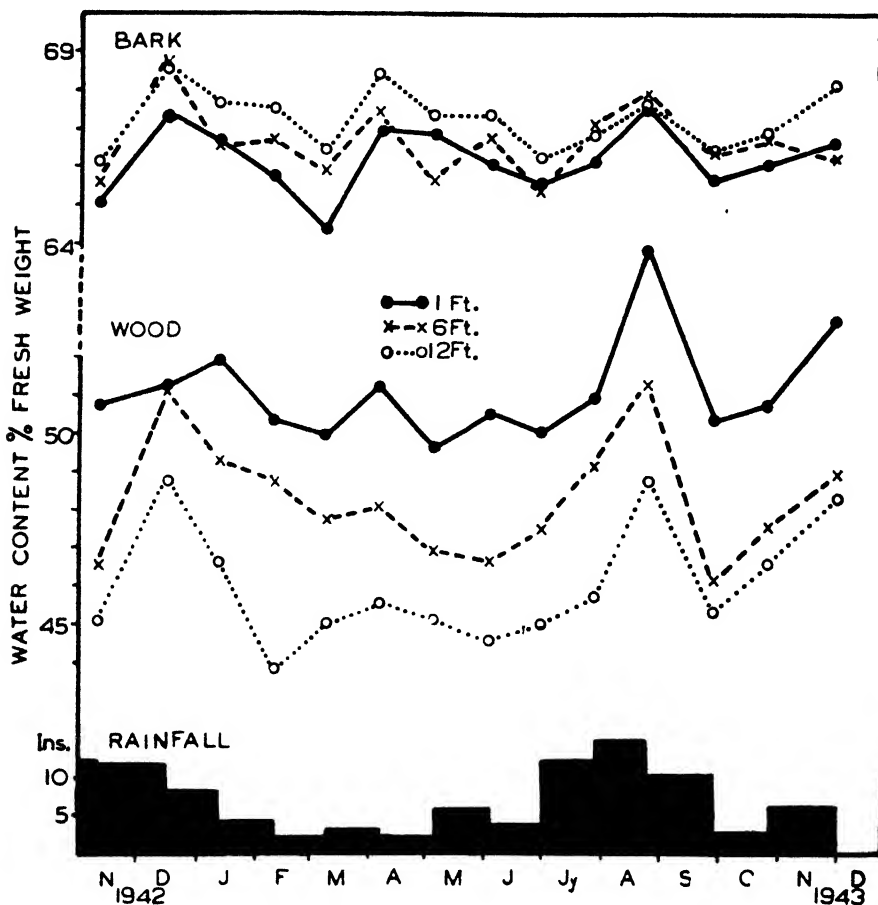


FIG. 1. The seasonal changes in water content of the bark and wood at three levels together with rainfall data.

The highest water content in the bark at the 12-ft. level might be accounted for by the cortex being relatively more extensive at this level than at 6 ft. or 1 ft., and in the absence of data for the water content of the cortex alone no definite statement can be made. The examination of transverse sections did not reveal any very great difference in the relative amount of cortex at the three levels, and it may be seen from a cross-section of the bark at the 6-ft. level (Fig. 8) that the amount of cortex is small compared with that of the phloem, so that the gradient of water content in the bark that the data indicate may be real. In a special sample in which the bark was divided tangentially into three approximately equal portions the water content of the outside

TABLE II

Net Percentage Variation of Constituents of Bark and Wood at Three Levels. (Constituents as gm. per 100 gm. Water)

Level.	Water content (% F.W.).		Alcohol-soluble material.		Non-carbo- hydrate alcohol-soluble material.		Sucrose.		Total reducing sugars.		Starch.	
	Bark.	Wood.	Bark.	Wood.	Bark.	Wood.	Bark.	Wood.	Bark.	Wood.	Bark.	Wood.
1 ft.	1.92	3.01	9.71	15.12	11.20	16.66	18.84	17.62	18.65	24.59	27.23	17.43
6 ft.	1.83	4.04	9.11	11.79	10.20	14.20	18.33	15.83	19.19	25.12	30.10	22.03
12 ft.	1.73	3.69	10.79	12.23	12.29	15.15	16.20	14.04	20.40	17.70	30.81	24.08

third (Table III) was intermediate in value both at the 6-ft. and 12-ft. levels, so that it does not seem that the gross water content of the bark is unduly affected by the water contained in the outer layers of the bark. In any case the gradient of water in the bark is small compared with that of the wood.

TABLE III

Composition of the Bark of the Cacao Tree Divided Tangentially into Three Equal Portions at Three Different Levels

Per cent. of fresh weight.						
	Water content.	Total reducing sugars.	Sucrose.	Alcohol-soluble material.	Non-carbo- hydrate alcohol-soluble material.	Per cent. of dry weight. Starch.
1 ft.						
Outside third	62.21	0.068	0.287	5.240	4.885	5.21
Middle third	65.96	0.098	0.717	5.670	4.855	6.98
Inside third	67.25	0.119	0.552	5.809	5.138	5.20
6 ft.						
Outside third	66.55	0.095	0.384	4.050	3.571	7.49
Middle third	65.83	0.129	0.538	4.454	3.787	10.29
Inside third	67.18	0.162	1.228	5.088	3.698	6.25
12 ft.						
Outside third	66.06	0.263	0.479	5.344	4.602	6.72
Middle third	65.70	0.258	0.617	5.520	4.645	8.95
Inside third	66.15	0.187	0.997	5.838	4.654	5.44

The correlation between water contents of bark and wood at corresponding seasons, considering the three levels together, is $+0.737$ ($P = 0.01$), indicating that the water contents of wood and bark are affected in a like manner by seasonal changes. The season correlation coefficients at each level are likewise significant and are $+0.603$, $+0.772$, and $+0.538$ respectively, for the 1-ft., 6-ft., and 12-ft. levels.

(b) *Alcohol-soluble material*

The drift in total alcohol-soluble material in the bark, expressed as the amount associated with 100 gm. water, is shown in Fig. 2. The trend is

closely similar at the three levels, the 1-ft. level having the highest values throughout, whereas the values at the 6-ft. and 12-ft. levels lie fairly close to one another. Three distinct peaks occur, viz. January–February, June, and October. These times approximate to, but do not exactly correspond with, the occurrence of vegetative flushes throughout the field. This point will be discussed later. There proved to be a significant seasonal change in total alcohol-soluble material and a very highly significant difference between levels (Table I). There is no interaction between seasons and levels.

In the wood, as in the bark, three peaks in the total alcohol-soluble material are evident at approximately the same times, although the one in June is not

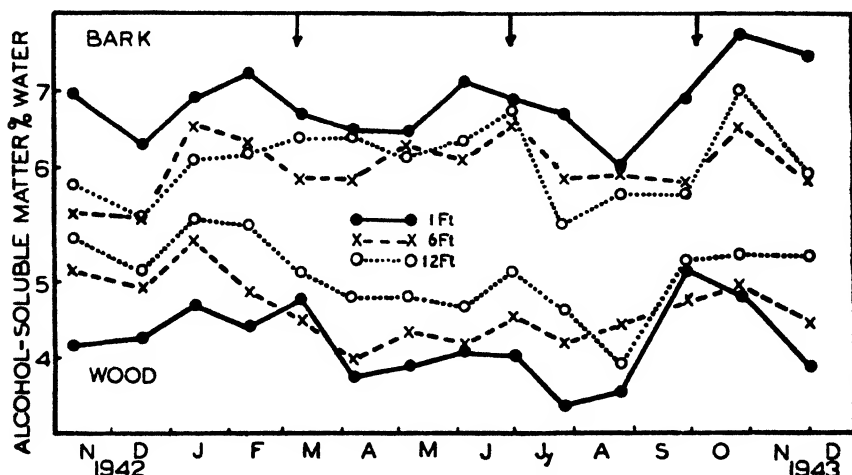


FIG. 2. The seasonal changes in total alcohol-soluble material of the bark and wood at three levels. In this and subsequent figures the downward-pointing arrows indicate the times of occurrence of vegetative flushes.

so marked. There appears to be a real decline in the amount of alcohol-soluble material present in the wood during the dry season. The gradient of total alcohol-soluble material is in the opposite direction to that in the bark. This is due to the non-carbohydrate component; the gradient of carbohydrate material is in the same direction. The term 'total alcohol-soluble material' includes a complex of substances both carbohydrate and non-carbohydrate in nature, and it is probable that, although some substances, e.g. sucrose, glucose, and fructose, are included in the fraction in both bark and wood, other components will be peculiar to either the bark or wood. For this reason it is probably not of much value to make a strict comparison between the amounts of this fraction in bark and wood. The seasonal changes in total alcohol-soluble material of the wood are very highly significant, as are also the differences between levels.

(c) *Non-carbohydrate alcohol-soluble material*

The values for this fraction have been calculated by subtracting total sugars (sucrose and total reducing sugars) from total alcohol-soluble material. The

values expressed on a basis of 100 gm. water are shown in Fig. 3. The trend is very similar to that of total alcohol-soluble material, and again the amounts in the bark are higher at the 1-ft. level than at the 6-ft. and 12-ft. levels, where the values are very similar. The seasonal variation is highly significant and the levels differences very highly significant. There is no significant interaction between seasons and levels.

The changes of this fraction in the wood are very similar to those of the total alcohol-soluble material, the values being highest at the 12-ft. level and

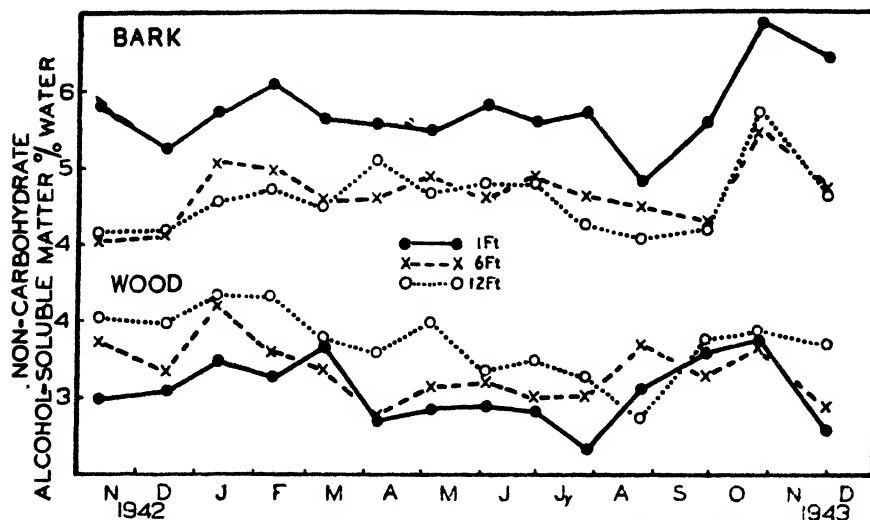


FIG. 3. The seasonal changes in non-carbohydrate alcohol-soluble material of the bark and wood at three levels.

lowest at the 1-ft. level. The seasonal changes and levels differences have a similar degree of significance to those in the bark (Table I). The season correlation coefficient between total sugars and non-carbohydrate alcohol-soluble material is not significant.

(d) *Sucrose*

The seasonal trend in sucrose values at the three levels in the bark are given in Appendix Table IV, where the values are expressed on a fresh-weight basis. The results, expressed as a concentration of sucrose per 100 gm. water, have been plotted in Fig. 4. The sucrose content is highest at the 12-ft. level and lowest at 1-ft., i.e. a positive gradient exists. At the 1-ft. level three distinct peaks are evident—in January, June, and September. The trend at the 6-ft. level is very similar, and at 12-ft. the June peak is very marked. It appears that the fall of sucrose concentration from the peak values is to a certain extent associated with the onset of vegetative flushing. General flushes over the whole field were recorded in March, June, and October (marked by downward-pointing arrows in Fig. 4), the two latter occasions corresponding with the time when peaks in sucrose concentration occur at the 1-ft. and 6-ft. levels.

The onset of the flushes causes a big drain on the carbohydrate reserves of the tree and a very sudden decline occurs; then the sucrose reserves accumulate until another flush takes place. The fall from the January peak value began some time before a flush was evident, and in this case the initial decrease may be due to the incidence of the dry season, but the variable demand of the crop

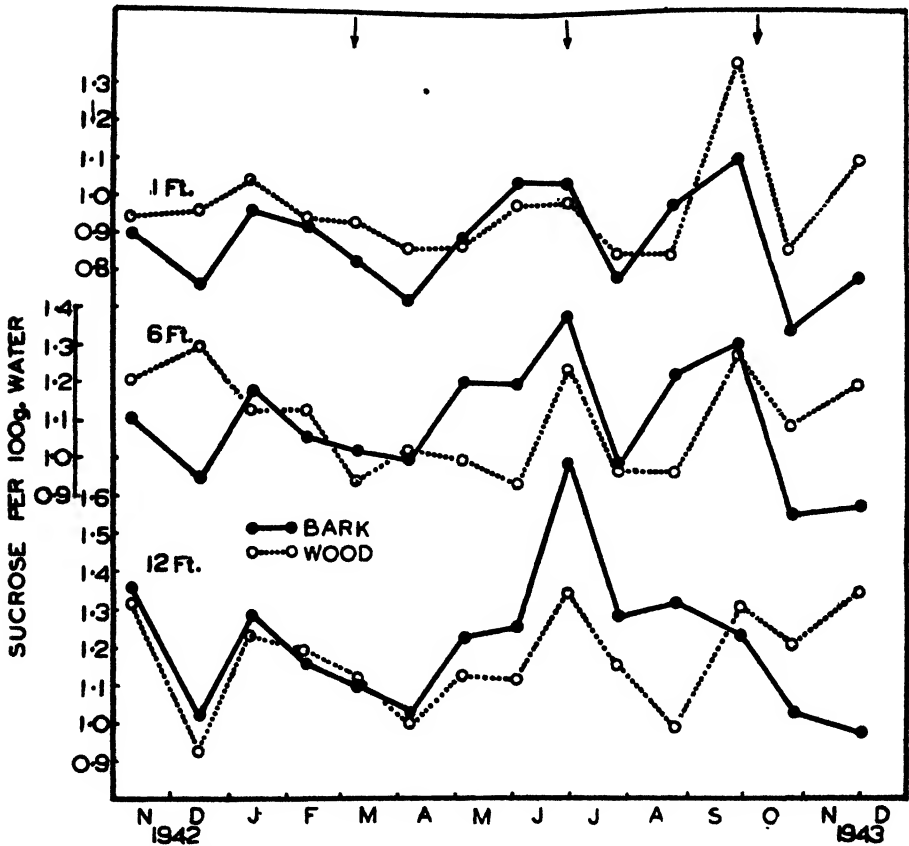


FIG. 4. The seasonal changes in sucrose of the bark and wood at three levels.

has also to be taken into account in considering the fluctuations of sucrose and other reserves. Only one of the peaks at the 12-ft. level could definitely be associated with a flush. The seasonal change of sucrose in the bark is highly significant and the difference between levels very highly significant. There is no significant interaction between seasons and levels.

The sucrose value for the three levels of the wood are plotted with the bark values (Fig. 4) for comparison. The trend in the wood is similar to the trend in the bark at any particular level and the maximal values occur at the same time as in the bark. The only marked difference between the trend in bark and wood is during the dry season where the values in the wood tend to decrease, especially at the 6-ft. level; this is possibly a drought effect. As

in the bark, there is also a positive gradient of sucrose in the wood. The fact that the gross concentration of sucrose in the wood was closely similar to that of the gross concentration in the bark must be largely coincidental since the sugar concentration is not the same in all parts of the bark (see Table III and p. 10) and probably not in all parts of the wood. It is of interest to compare the seasonal trend of the sucrose *gradients* in the bark and wood. The gradients between the 12-ft. and 1-ft. levels have been calculated and are shown in Fig. 5. The gradient in the bark is greater than in the wood throughout, but there is a marked similarity between the two curves except in the dry season when the bark gradient increases while that of the wood decreases slightly.

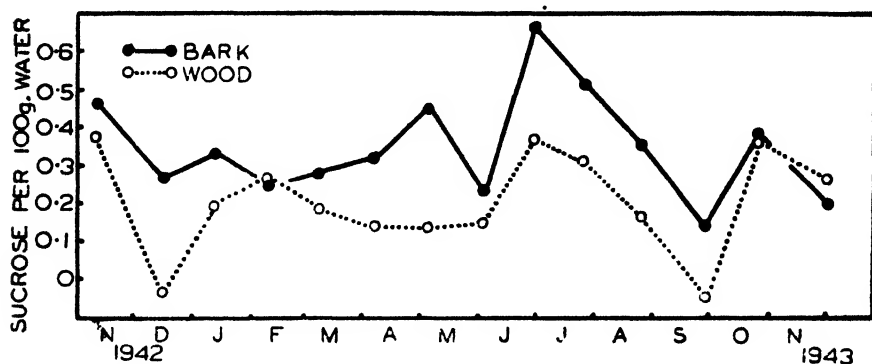


FIG. 5. The seasonal changes in sucrose gradient between the 12-ft. and 1-ft. levels in bark and wood.

There was a slight negative gradient in the wood on two occasions (in December and in the following September).

The fact that the sucrose gradient in the wood runs fairly parallel to that in the bark is in harmony with the view (Mason and Maskell, 1928) that the bark is the main channel of sucrose transport, but that horizontal diffusion of sucrose from bark to wood occurs. They showed that the rate of movement from bark to wood is probably not outside the range of physical diffusion. Since rate of diffusion is proportional to concentration of sucrose in the bark it is to be expected that the wood will reflect the changes in gradient exhibited by the bark. This is also supported by the fact that the average sucrose values in the bark over the whole season at each of the three levels are significantly correlated with the average sucrose values in the wood at corresponding levels ($r = +0.9998$, $P = 0.01$).

An additional point exhibited in Fig. 5 is also worthy of note. There is a consistent rise in the value of the gradient in the bark during the dry season, but from the commencement of the wet season (June) a fairly rapid decline occurs. This is no doubt connected with the different light conditions in the two seasons. There is also an indication of a secondary increase in gradient in October when a short dry spell occurred (see rainfall data in Fig. 1).

Both seasonal and levels differences in the wood are highly significant (Table I). There is no interaction between seasons and levels. Although the sucrose gradients of bark and wood are fairly parallel, the season correlation coefficient between sucrose concentrations of the bark and wood (sum of three levels) is not significant ($r = +0.316$), but considering the three levels

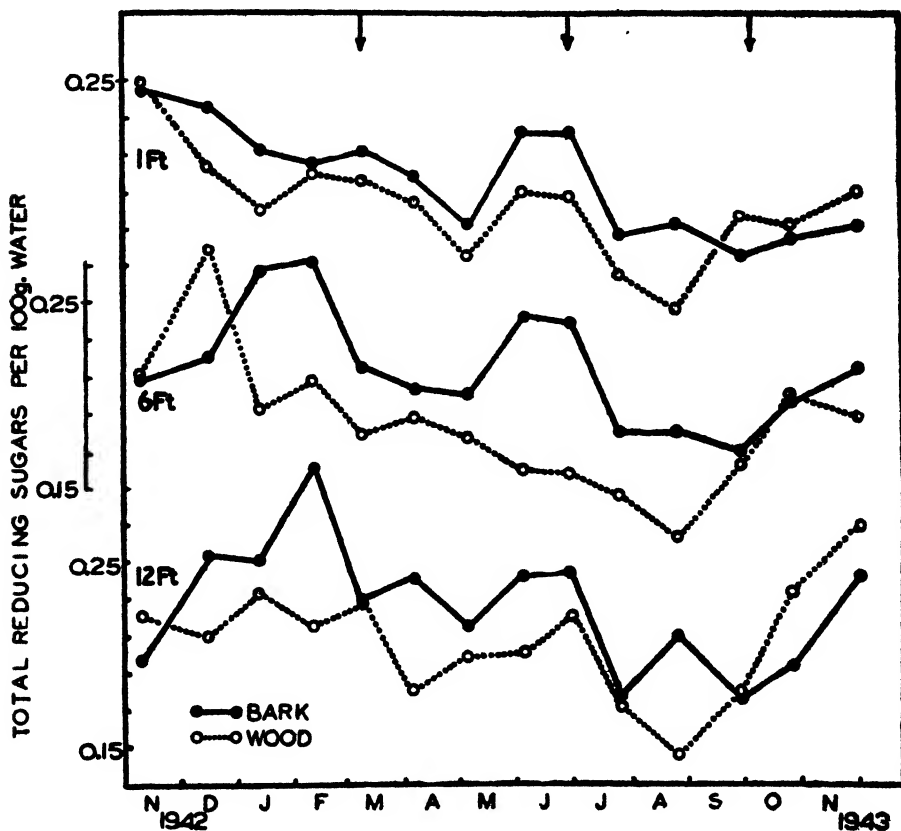


FIG. 6. The seasonal changes in total reducing sugars of the bark and wood at three levels.

separately, the correlation coefficients at 1 ft., 6 ft., and 12 ft. respectively are $+0.571$, $+0.054$, and $+0.386$, indicating a significant correlation at the 1-ft. level only.

(e) Total reducing sugars

The changes in concentration of total reducing sugars in the bark, from season to season, is shown in Fig. 6. At the 1-ft. level there is a net decline during the period of observation with a marked peak in June. At the 6-ft. level two peaks are evident—in January and June—and although there is subsequently a decline until September, the values finally increase again to

attain the initial value at this level. The trend at the 12-ft. level is somewhat similar to the 6-ft. level. Both the seasonal changes and levels differences are highly significant.

The drift of total reducing sugars in the wood has a general similarity to that in the bark, especially at the 1-ft. level. The decline from the peaks as

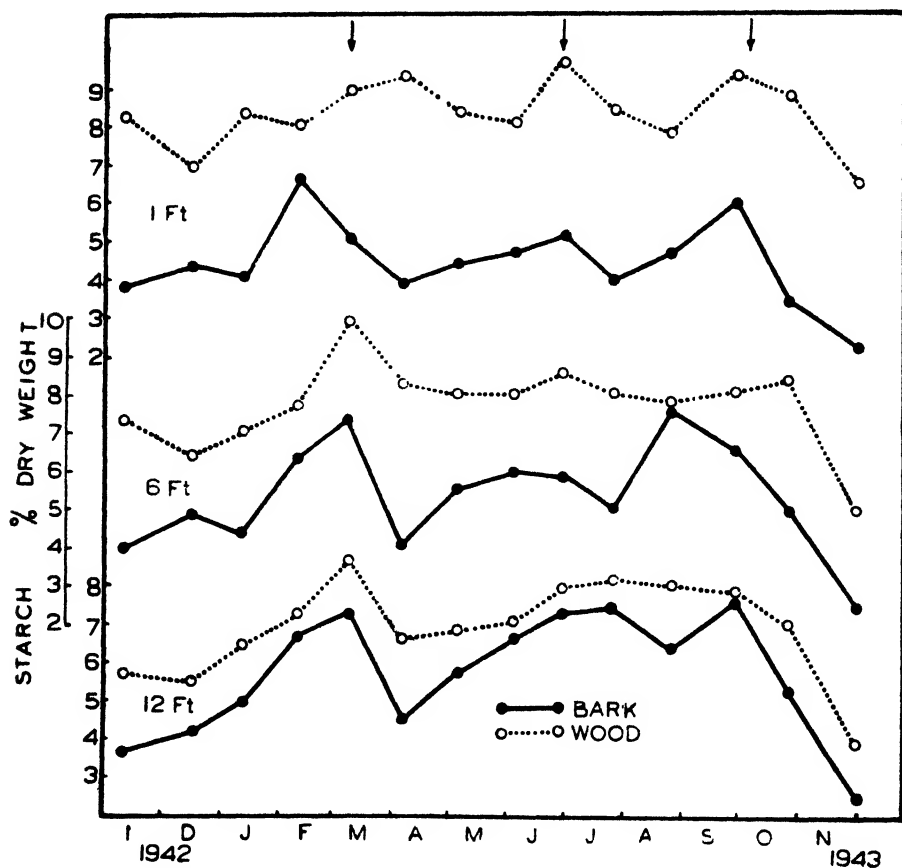


FIG. 7. The seasonal changes in starch of the bark and wood at three levels.

in the bark again appears to be partly associated with flushing. The seasonal changes and levels differences are very highly significant (Table I). The season correlation coefficient between reducing sugars of bark and wood (sum of three levels) is significant ($r = +0.617$), but the values for the 1-ft., 6-ft., and 12-ft. levels respectively are $+0.735$, $+0.334$, and $+0.420$, being significant as 1 ft. only as in the case of sucrose.

Separate analyses of glucose and fructose were made throughout and the proportions of these two sugars were approximately equal in all samples, and have not been separately recorded. The concentration of total reducing sugars was much less than that of sucrose both in bark and wood.

(f) Starch

Fig. 7 and Appendix Table VI show the seasonal trend of starch in the bark (on a dry-weight basis). At the 1-ft. level three distinct peaks occur, viz. in February, June, and September—and the decline from these peaks is much more clearly related to the occurrence of vegetative flushing than in the case of sucrose and total reducing sugars. The trends in starch content at the 6-ft. and 12-ft. levels are very similar, but the first peak occurred about a month later at these levels. The starch content of the bark is highest at the 12-ft. level and lowest at the 1-ft. level. Seasonal changes and levels differences are very highly significant with no interaction between seasons and levels.

The fluctuations of starch in the wood are plotted on the same graph as those of the bark (Fig. 7) in order to compare the relative trends. At 1 ft. the percentage of starch in the wood is considerably greater than in the bark. There is no marked decrease in starch content in the wood at 1 ft. at the time of the first flush, suggesting that the starch reserves at this level are not utilized as readily as those higher in the tree. The starch reserves of the wood at 6 ft. do not show any very marked fluctuation, but there is an obvious peak in March. At the 6-ft. level the percentage of starch in wood and bark approach one another more closely than at the 1-ft. level. At the 12-ft. level the trend of starch in the wood is similar to that in the bark and the values approach one another, although the bark values are always below those of the wood. The starch gradient is opposite in the bark and wood. A feature of the starch trend in bark and wood at all levels is the rapid decrease in the last two months, which coincides with the incidence of a flush and a comparatively large (increasing) crop on the trees. The values in the bark at the 6-ft. level, for instance, decrease from 6.60 to 2.43 per cent.

The seasonal changes and levels differences have a similar degree of significance to those in the bark. The interaction between seasons and levels is not significant. Taking the three levels together the season correlation coefficient between the starch of bark and wood is $+0.787$ ($P = 0.01$) and the three levels separately the values are $+0.423$, $+0.700$, and $+0.936$ for the 1-ft., 6-ft., and 12-ft. levels respectively, the last showing a very high correlation. When the starch is expressed as amount associated with 100 gm. water there is no significant difference between levels in the wood, i.e. the same amount of starch is associated with 100 gm. water at all levels (Table I), but there is a marked gradient in the bark.

Starch in the wood fluctuates much less markedly than in the bark at all three levels (see data for net percentage variation in Table II), and this may mean that the starch reserves of the bark are drawn upon sooner than those of the wood. Alternatively, since the wood was sampled as a whole, if the starch reserves in the outer layers of the wood are first drawn upon, bigger fluctuations in those layers would be masked by the amount of starch remaining in the other parts.

From the data available it is possible to investigate the seasonal relationship between constituents. The season correlation coefficients between the

following pairs of constituents have been calculated: total reducing sugars and sucrose; total reducing sugars and starch; sucrose and starch. In the first case the correlation coefficient is low both in bark and wood but just approaches significance at the 12-ft. level in the wood ($r = +0.526$). In the second case the correlation coefficients are all insignificant at all levels. In the third case the correlation at the 6-ft. level in the bark is just significant, while at the other levels in the bark and in all levels in the wood the values are insignificant.

NET PERCENTAGE VARIATION OF CONSTITUENTS OF BARK AND WOOD

In order to obtain an idea of the relative variation of constituents over the whole season the net percentage variation of each constituent at each level has been computed by dividing the standard deviation of each constituent by the mean. These values are shown in Table II. The variation in alcohol-soluble material (per 100 gm. water) in the wood is at all levels more variable than in the bark. This also applies to the non-carbohydrate alcohol-soluble material. In the case of sucrose, however, it is more variable in the bark. Total reducing sugars are more variable in the wood except at 12 ft. The variation of starch (per 100 gm. water) is much greater in the bark than in the wood.

RADIAL DISTRIBUTION OF CONSTITUENTS IN THE BARK

In the foregoing account all the values discussed were obtained by the gross analysis of the bark or the wood and some idea of the distribution of the carbohydrates in the bark was obtained from special samples taken about 6 weeks after the termination of the main experiment. The samples were taken in the usual manner from ten trees at three levels. Samples from each level were bulked and the bark was divided tangentially, as nearly as possible, into three equal portions—outside, middle, and inside zones. Determinations of water content, alcohol-soluble material, sucrose, total reducing sugars, and starch were made on these samples. The results are summarized in Table III.

The greatest difference in water content between zones is at the 1-ft. level, the inside third having the highest value. There is no very great difference in water content between the three zones at the 6-ft. and 12-ft. levels. The total reducing sugar content at the 1-ft. and 6-ft. levels is highest in the inside third and lowest at the outside. The reverse is the case at the 12-ft. level. The sucrose content at all levels is lowest at the outside. It is especially high in the inside third of the 6-ft. level. The total alcohol-soluble material is in all cases lowest at the outside and highest inside. The non-carbohydrate alcohol-soluble material is practically the same in each zone at any one level. The starch contents of the three zones at the 1-ft. level are fairly similar but highest in the middle. The higher value in the middle zone at the 6-ft. level is more noticeable than at the 1-ft. level. At 12 ft. the starch content is also greatest in the middle zone. Thus there is a marked difference between zones for most constituents. In order to see if the distribution of the various con-

stituents in the three parts of the bark could be related to the tissue distribution, transverse sections of the bark at the three different levels were examined. Fig. 8 is a diagrammatic representation of such a transverse section at the 6-ft. level. It may clearly be seen that starch (represented by dots) is most

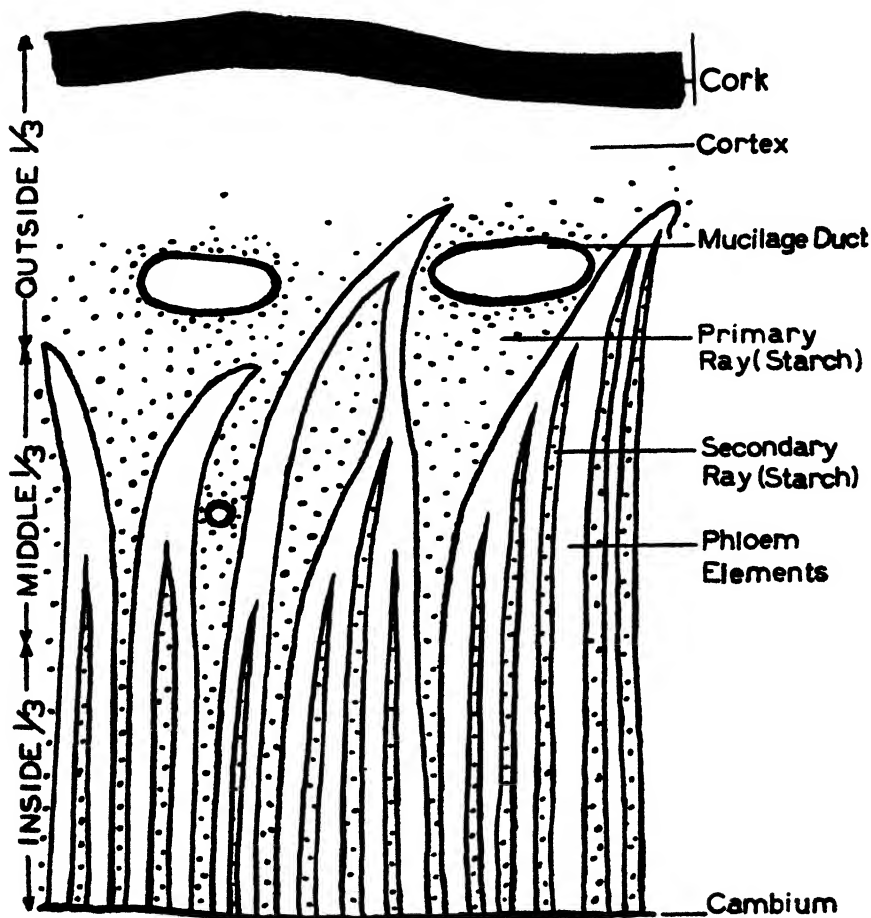


FIG. 8. A portion of a transverse section of the bark of the cacao tree, showing tissue distribution. The approximate distribution of starch (dots) is also shown.

plentiful in the middle zone where the medullary rays widen out into the cortex. The outer cortex, forming the major part of the outside zone, is completely free from starch. Microscopical examination of the 1-ft. and 12-ft. levels confirm the results of the starch analysis, viz. that starch is more abundant at the 12-ft. level than at the 1-ft. level. Large single crystals of calcium oxalate occur in the medullary ray cells. The analyses for the three zones considered in conjunction with the tissue distribution suggest that the sucrose is mainly located in the phloem elements—a fact previously established by Mason and Maskell (1928) in the case of the cotton plant—while the starch

is mainly located in the ray tissue. Mason and Maskell found that in the bark of the cotton plant reducing sugars were opposite in radial gradient to sucrose. In the present case this is true at the 12-ft. level, but the gradient is in the same direction as that of sucrose at the 1-ft. and 6-ft. levels.

SEASONAL CHANGES IN FRUITING INTENSITY

One of the objects of the experiment was to attempt to estimate the effect of the crop on the carbohydrate reserves in the tree. For this purpose it is necessary to have information concerning the changes in carbohydrate demand of the crop from season to season which requires knowledge of the number and age of fruits on the trees at any time. This was obtained in the present experiment by counting the number of fruits falling into particular size classes on each of the twenty trees sampled on each occasion. The age limits of each size class were estimated from results obtained in a previous investigation (Humphries, 1943, p. 47) and are as follows: very small, 0-73 days; small, 78-84 days; medium, 87-107 days; full, 122-43 days; ripe (or diseased), 143-70 days. By combining this information with data on carbohydrate content of the wall and pulp of the cacao fruit (*loc. cit.*, pp. 53 and 56) the *average* carbohydrate content of a fruit in each class may be calculated. The carbohydrates concerned are starch, sucrose, glucose, and fructose. Fat, which is especially abundant in the seeds of the ripe fruit, was also included in the estimate of carbohydrate. The total carbohydrate content of the fruits of a ten-tree sample affords an estimate of the carbohydrate uptake by the crop at any time. No figures were available for the content of cellulose and other structural carbohydrates so that the carbohydrate uptake figures include only labile carbohydrates and fat (formed from labile carbohydrates). This estimate of crop carbohydrate assumes that the average size of a fruit (and therefore its carbohydrate content) in a given size class does not change from season to season, while it is known (e.g. Humphries, 1940) that fruits developing during the dry season tend to be smaller than those growing during the wet season. It is thought, however, that this fact will not affect the estimate to any great extent. Table IV shows the number of fruits of various ages present on the trees at successive sampling occasions. In this table the total carbohydrate content (as glucose) of the crop on the ten trees constituting each sample is also given. The seasonal trend in carbohydrate demand of the crop is also illustrated in Fig. 9, where the data for the A and B samples are plotted. The total dry weight of the crop on different occasions was also calculated as an alternative measure of crop size and these figures appear in Table IV.

The seasonal trend in carbohydrate constituents (Figs. 2-7) show little relationship to the seasonal fluctuations in crop carbohydrate demand. For instance, during the period January to August the crop is comparatively small but large changes in reserve occur. There appears to be little doubt that these fluctuations are connected with the vegetative condition of the trees. The carbohydrate reserves increase until a vegetative flush occurs when the reserves

TABLE IV
Seasonal Trend in Fruiting Condition of the Experimental Trees

Date.	Sample no.	Very small.	Number of fruits.					Crop units (carbohydrate as gm. glucose).	Crop units (total dry weight, gm.).
			Small.	Medium.	Full.	Ripe.	Diseased.		
1942									
Nov. 10	1A	36	11	7	11	11	7	818.96	2945
	1B	56	13	18	11	1	8	585.61	2545
Dec. 16	2A	40	1	11	7	7	34	1491.06	4838
	2B	16	13	9	7	3	36	1425.21	4675
1943									
Jan. 12	3A	8	2	2	4	3	12	562.96	1808
	3B	2	18	13	10	12	13	1053.32	3727
Feb. 9	4A	17	4	0	2	6	4	360.73	1178
	4B	22	7	4	2	3	4	286.19	1081
Mar. 9	5A	58	8	1	5	5	9	555.41	1967
	5B	5	4	1	2	9	3	427.71	1367
Apr. 6	6A	22	4	11	4	2	4	319.83	1326
	6B	12	2	3	3	1	2	166.80	654
May 5	7A	2	4	3	7	6	2	399.36	1406
	7B	2	0	3	8	0	5	318.43	1146
June 3	8A	42	9	12	12	4	5	571.55	2329
	8B	13	0	3	10	8	2	515.56	1788
June 29	9A	44	0	0	3	14	1	538.33	1742
	9B	69	0	1	8	8	0	412.95	1543
July 27	10A	175	14	3	2	16	0	592.60	2398
	10B	40	3	1	0	3	0	107.45	462
Aug. 24	11A	40	12	10	8	2	0	268.78	1352
	11B	56	10	4	1	0	0	51.07	483
Sept. 28	12A	92	40	26	22	16	8	1326.52	5444
	12B	56	26	18	17	0	4	545.43	2668
Oct. 27	13A	179	54	24	46	26	56	3625.46	12949
	13B	145	43	39	57	10	48	3113.77	11749
Dec. 1	14A	27	4	2	17	41	10	1951.56	6193
	14B	32	4	5	19	16	13	1299.75	4383

are transported to the actively growing shoots resulting in a rapid decrease in the amount in bark and wood. When the young leaves have attained their full size—a period of 4 or 5 weeks (Humphries, 1939)—there is export of carbohydrates and the reserves in the bark and wood are built up again. It was shown in another investigation (Humphries and McKee, 1943) that the depletion of reserves during active growth of a new flush is also evident in the bark and wood immediately behind the flush and that the reserves begin to increase again as soon as the flush has completed its extension growth. It may be assumed, therefore, that the carbohydrate reserves are depleted by a flush from the tips of the branches to the base of the trunk. In the absence of data concerning the carbohydrate changes in the roots it is not possible to say whether the reserves here are also affected, but it is probable that this is the case.

THE EFFECT OF CHANGES IN SIZE OF CROP ON THE CARBOHYDRATE RESERVES

An unexpected feature of the sampling was that on 13 out of the 14 sampling occasions, the A crop was larger than the B crop (see Fig. 9). Since the trees to be sampled were chosen at random before the commencement of the experiment, this distribution must be due to chance. It is also apparent from a consideration of the alcohol-soluble and carbohydrate data (Appendix Tables II to VI) that the values of the B samples are usually greater in magnitude than the corresponding A samples. At first it was thought that

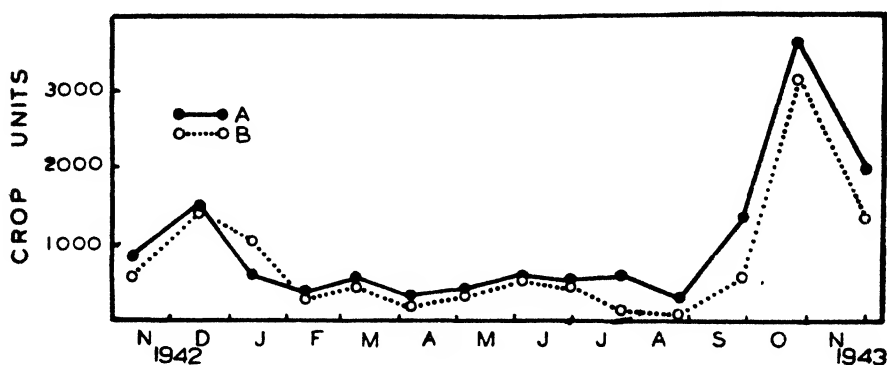


FIG. 9. Seasonal changes in fruiting intensity of the A and B samples. Crop units are expressed in terms of gm. carbohydrate (as glucose) in the crop at any given time.

this might be due to diurnal variation since the B samples were always collected $1\frac{1}{2}$ hours later than the A samples, but further consideration suggested that it was concerned with the difference in magnitude of the crop on the two sets of trees.

In order to compare the changes in crop carbohydrate with changes in the carbohydrate constituents of bark and wood it is necessary to eliminate the considerable seasonal effects (mainly due to vegetative flushing). This has been attempted by means of an analysis of covariance. The 14 pairs of concomitant observations on crop carbohydrate and carbohydrate constituents of bark and wood afford 27 degrees of freedom having as components the seasonal variation (13 degrees of freedom) and a residue with 14 degrees of freedom. This residual component is a measure of the relationship between the carbohydrate component under examination and the carbohydrate of the crop when the extraneous seasonal effects have been removed, i.e. the variation between trees sampled in the same day which may be ascribed to effects due to factors such as soil or genetic constitution. This procedure allows of a comparison between the crop carbohydrate and the carbohydrate of the bark or wood at any level or the bark or wood as a whole as desired. Each of the constituents have in this way been compared in turn with the crop carbohydrate and the appropriate correlation coefficients calculated from the

residual covariance and the residual variances of the constituent and crop carbohydrate. The complete data are too extensive to be presented in full, but the results obtained in the case of sucrose and non-carbohydrate alcohol-soluble material which exhibit points of special interest are given in Table V. It will be observed that at all levels in the bark the total, seasons, and residual covariance values for sucrose are negative. The correlation coefficients calculated from the total variation are all significant except at the 1-ft. level.

TABLE V

Analysis of Covariance of Sucrose and Non-carbohydrate Alcohol-soluble Material of Bark and Wood and of Crop Carbohydrate

Variation due to:		Sucrose variance (S.S.).	Covariance: Sucrose x Crop carbohydrate (S.P.).	Correlation coefficient (r).	Non-carbohydrate alcohol-soluble material variance (S.S.).	Covariance: N.C.A.S.M. x Crop carbohydrate (S.P.).	Correlation coefficient (r).
Bark							
Sum 3 levels	Total (T)	7.004	-6138.86	-0.524*	57.15	+14443.6	+0.431†
	Season (S)	5.119	-5187.04	-0.531	35.55	+16484.8	+0.640†
	Residual (R)	1.885	-951.82	-0.702*	21.60	-2041.2	-0.445
1 ft.	T	0.7213	-1707.46	-0.376	11.27	+6478.9	+0.388†
	S	0.4811	-1410.56	-0.471	6.37	+7838.4	+0.719*
	R	0.2402	-296.90	-0.614†	4.90	-1359.5	-0.622*
6 ft.	T	1.0786	-2311.23	-0.502*	6.17	+2705.6	+0.246
	S	0.6509	-1945.63	-0.558†	3.66	+3217.6	+0.389
	R	0.4277	-365.60	-0.566†	2.51	-512.0	-0.327
12 ft.	T	1.0725	-2121.24	-0.462†	8.67	+5262.1	+0.443†
	S	0.9002	-1830.43	-0.446	5.15	+5432.7	+0.554†
	R	0.1723	-290.81	-0.710*	3.52	-170.6	-0.092
Wood							
Sum 3 levels	T	4.823	+1022.58	+0.105	37.01	+6761.0	+0.251
	S	2.811	+1731.56	+0.239	26.69	+8227.6	+0.369
	R	2.012	-708.98	-0.506	10.32	-1466.6	-0.462
1 ft.	T	0.7713	-38.91	-0.010	7.10	+3367.3	+0.285
	S	0.4781	+266.22	+0.089	4.72	+3822.4	+0.407
	R	0.2932	-305.13	-0.571†	2.38	-455.1	-0.299
6 ft.	T	0.8183	+633.82	+0.158	6.07	+1436.2	+0.132
	S	0.4943	+894.85	+0.295	3.93	+1551.8	+0.181
	R	0.3240	-261.03	-0.465	2.14	-115.6	-0.080
12 ft.	T	0.7290	+427.77	+0.133	8.45	+1960.7	+0.152
	S	0.4782	+570.99	+0.191	4.79	+2856.1	+0.302
	R	0.2508	-143.22	-0.290	3.66	-895.4	-0.475

* Significant at $P = 0.01$.

† Significant at $P = 0.05$.

The corresponding crop carbohydrate variance values are:

Total	19,626,524.73
Season	18,651,784.51
Residual	974,740.22

While all the season correlation coefficients are negative they fail to reach significance except at the 6-ft. level. The correlations calculated from the residual variances are significant at all levels. In the wood the total and

seasons covariance values in the case of sucrose are all positive except in one instance. All the residual covariance values are, however, negative and the correlation coefficient is significant at 1 ft. The non-carbohydrate alcohol-soluble material data show somewhat opposite trends especially in the bark. Thus the total and seasons covariance values are positive both in bark and wood while the residual covariance values are negative. The correlation coefficients are positive and significant for both total and seasons variation in the bark, while the negative residual correlation at the 1-ft. level in the bark is also significant. None of the correlation coefficients in the wood proved to be significant. While sucrose exhibits a negative seasonal correlation with crop carbohydrate which approaches significance at $P = 0.05$, the non-carbohydrate alcohol-soluble material exhibits a positive seasonal correlation with crop which is significant. In both cases, however, the residual correlation is negative and significant at all levels in the case of sucrose and at the 1-ft. level in the case of non-carbohydrate alcohol-soluble material. It therefore appears that sucrose reserves are depleted by an increasing crop while the non-carbohydrate fraction fluctuates directly with the crop. This fraction which consists in part of nitrogenous substances appears to be mobilized according to the crop demand.

For the other constituents only the correlation coefficients of the residual components are presented (Table VI). The alcohol-soluble material of the

TABLE VI

Correlation Coefficients Calculated from Residual Components of Constituents (per 100 gm. Water) of Bark or Wood and Residual Component of Crop

Constituent.		Sum 3 levels.	1 ft	6 ft.	12 ft	Sum 3 levels.	1 ft.	6 ft.	12 ft.
Alc.-sol. material	{ Bark	-0.588*	-0.070†	-0.443	-0.353	-0.555*	-0.603*	-0.461	-0.316
	{ Wood	-0.584*	-0.399	-0.313	-0.546*	-0.666†	-0.381	-0.452	-0.611*
Non-carb. alc.- sol. material	{ Bark	-0.445	-0.622*	-0.327	-0.092	-0.388	-0.531*	-0.344	-0.038
	{ Wood	-0.462	-0.299	-0.080	-0.475	-0.488	-0.222	-0.140	-0.545*
Sucrose	{ Bark	-0.702†	-0.614*	-0.566*	-0.710†	-0.712†	-0.664†	-0.577*	-0.664†
	{ Wood	-0.506	-0.571*	-0.465	-0.290	-0.512	-0.584*	-0.500	-0.256
Total reducing sugars	{ Bark	-0.304	-0.210	-0.281	-0.297	-0.263	-0.169	0.271	-0.242
	{ Wood	+0.087	-0.004	+0.144	+0.078	+0.081	-0.022	+0.162	+0.058
Starch	{ Bark	-0.120	-0.023	-0.177	-0.057	-0.108	-0.110	-0.201	+0.013
	{ Wood	-0.315	-0.158	-0.404	-0.220	-0.318	-0.194	-0.420	-0.197
Starch (% D.W.)	{ Bark	+0.026	+0.208	-0.125	+0.045	+0.069	+0.249	-0.133	+0.133
	{ Wood	-0.272	-0.132	-0.386	-0.140	-0.273	-0.157	-0.380	-0.111

In the left-hand half of the table a comparison has been made between constituent and crop carbohydrate; in the right-hand half, crop dry weight is used as a measure of crop size.

* Significant at $P = 0.05$.

† Significant at $P = 0.01$.

bark shows a correlation coefficient of -0.670 ($P = 0.01$) at the 1-ft. level, while r is not significant at the other two levels, but the three levels considered together have a correlation coefficient of -0.588 ($P = 0.05$). In the wood the alcohol-soluble material has a significant negative correlation at 12-ft. and the three levels considered together also have a significant negative correlation. The correlations of the residual components of starch and total reducing sugars with crop are low and non-significant both in bark and wood.

There is thus a significant negative correlation between certain constituents, notably sucrose, of the bark and wood and carbohydrate content of the crop. It appears that the trees with the greater content of soluble carbohydrate are potentially capable of bearing as large a crop as those with the lesser content of carbohydrate. This suggests that the photosynthetic mechanisms of the two sets of trees are equally efficient and some other factor is responsible for limiting the size of the crop in one set. From data obtained in the experiment this limiting factor appears to be *potassium*, but fuller discussion of this question will be deferred until seasonal changes in mineral constituents are presented in a later paper.

In the foregoing account the total (labile) carbohydrate of the crop has been correlated with the carbohydrate constituents (and the non-carbohydrate constituents of the alcohol-soluble fraction) in turn. When mineral constituents are considered it will be desirable to have a common basis on which correlation coefficients between carbohydrate constituent and crop on the one hand and mineral constituent and crop on the other may be compared. The total dry weight of the crop, composed of carbohydrates and mineral substances, appeared to be the most satisfactory basis. The residual correlations between the carbohydrate constituents of the bark and wood and total dry weight of crop have been calculated and are shown in the second half of Table VI. It will be observed that they are in most cases of the same order of magnitude as obtained by the use of total crop carbohydrate.

The correlation coefficients presented in Table VI represent the relationship of a particular component to the crop carbohydrate in the presence of the other components, and it is of interest to calculate the partial coefficients in order to see if the general picture so far obtained is substantially altered. For this purpose the four variables, crop carbohydrate, sucrose (per cent. water), starch (per cent. water), and total reducing sugars (per cent. water), at the 12-ft. level of the bark have been considered, and the second-order correlations are as follows:

$$r_{12\cdot34} = +0.023 \qquad r_{14\cdot23} = +0.086$$

$$r_{13\cdot24} = -0.678 \ (P = 0.02) \qquad r_{24\cdot13} = +0.042$$

$$r_{23\cdot14} = +0.071 \qquad r_{34\cdot21} = +0.419$$

1 = crop carbohydrate, 2 = starch (per cent. water), 3 = sucrose (per cent. water), 4 = total reducing sugars (per cent. water).

The only significant partial correlation coefficient is between crop carbohydrate and sucrose, eliminating starch and total reducing sugars; thus there is no addition to the information supplied by the total correlations.

DISCUSSION

The existence of significant negative correlations between sucrose at all three levels of the bark and crop carbohydrate or crop dry weight (after elimination of seasonal effects) suggests that the bark is the main seat of sucrose storage, although a significant correlation at the 1-ft. level in the wood

suggests that the wood is concerned also, but not to the same extent, and may merely be an indication of sucrose storage in the wood of the root. That the bark and wood sucrose do not march together is indicated by their low seasonal correlation ($r = +0.316$, Table I); only at the 1-ft. level was the correlation significant ($r = +0.571$).

The sequence of changes in the carbohydrate reserves of a tropical tree such as cacao appears at first sight to be markedly different from that occurring in temperate trees. In the latter, in general, the reserve materials stored in the parenchyma of the trunk are largely mobilized in the spring and utilized to support growth of the young shoots. When the leaves produce carbohydrates in excess of requirements of vegetative and fruit growth, reserves again accumulate. Starch may be converted during the winter rest period into soluble carbohydrates (e.g. Gibbs, 1940). This phenomenon is apparently in response to a low temperature and may be reversed when warmer conditions prevail. In the case of the cacao tree, however, there are alternating maxima and minima in the carbohydrate reserves which are undoubtedly related to the vegetative condition of the tree. At each flush cambial activity is initiated over the whole tree, and this in turn is apparently the signal for hydrolysis of starch reserves both in the bark and wood and the resulting sugars are transported to the actively growing regions. So that each period of bud activity initiates the same sequence of changes which commences in a temperate tree with the unfolding of buds in the spring, and this sequence is repeated several times a year in the cacao tree.

The case of the cacao tree is also somewhat different from that of a temperate tree because there is usually some demand on the reserves of the tree by a developing crop all the year round (see Fig. 9). Whereas this demand only attains one maximum in the case of temperate trees there may be two maxima in the cacao tree under Trinidad conditions—one in November–December from fruit formed in June–July and another in April–May. The fruits formed at intermediate times suffer from competition from earlier-formed fruits and may wilt. The essential difference between temperate fruit-trees and the cacao tree is that in the latter there is no resting period but cycle is succeeded by cycle. There is evidence that these cycles are initiated by periods of high maximum temperature (Humphries, 1944a). The data suggests that vegetative flushing makes a much greater demand than the developing crop on the reserves of the tree.

The evidence here presented points to some factor other than carbohydrate which has the effect of limiting size of crop. From evidence already considered (Humphries, 1943, 1943a, 1944) it was concluded that wilting of young cacao fruits was due to competition for nutrients and the present investigation suggests that carbohydrate is not concerned in this competition.

SUMMARY

The seasonal changes in water content, alcohol-soluble material, non-carbohydrate alcohol-soluble material, sucrose, total reducing sugars, and

starch in the bark and wood of the cacao tree at levels of 1 ft., 6 ft., and 12 ft. from the base of the trunk have been followed over a period of 14 months.

There was a significant seasonal variation in all constituents and the differences between levels was also significant. There was no significant interaction between seasons and levels in the case of any constituent. The gradients of water content, alcohol-soluble material, non-carbohydrate alcohol-soluble material, and starch were opposite in the bark and wood. The gradients of sucrose and total reducing sugars were in the same direction.

Maximal values of alcohol-soluble material, sucrose, total reducing sugars and starch occur immediately before a vegetative flush and decline is associated with utilization for new growth.

The season correlation between water content of bark and wood was significant at all levels. The season correlation coefficient between sucrose content of bark and sucrose content of wood was significant only at the 1-ft. level. This applied also to the case of total reducing sugars. The season correlation coefficient between starch of bark and starch of wood was significant at all three levels, being particularly high at the 12-ft. level.

In a special sample taken to investigate the radial distribution of constituents in the bark it was found that sucrose content was highest in the inside of the bark while starch was highest in the middle. This is in harmony with the view that sucrose is located mainly in the phloem elements while starch is located mainly in the medullary ray cells.

The seasonal change in crop was followed by counting the number of fruits of various sizes present on the trees at any time. An estimate of the crop size was obtained from the aggregate carbohydrate content of the fruits. Dry matter of the crop was also used as an alternative estimate. By means of an analysis of covariance between a given constituent of the bark or wood and crop size, seasonal effects were eliminated and the relationship between amount of constituent and crop size was studied. There was found to be a significant negative correlation between crop and sucrose content at all levels of the bark and at the 1-ft. level of the wood. It was concluded that carbohydrate content of the trees with the smaller crop was not limiting the size of the crop and that some other factor was concerned.

The points of resemblance and difference between the behaviour of temperate trees and a tropical tree such as the cacao tree are briefly discussed.

The author is grateful to Dr. S. G. Stephens for help and advice in the early stages of the experiment, and to Dr. E. J. Maskell for stimulating discussions which have been of considerable assistance in the critical examination of the data.

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APPENDIX, TABLE I

Seasonal Trend in Moisture Content as a Percentage of Fresh Weight of Bark and Wood at Three Levels

Sampling date.	Bark.						Wood.					
	1 ft.		6 ft.		12 ft.		1 ft.		6 ft.		12 ft.	
	A	B	A	B	A	B	A	B	A	B	A	B
Nov. 10	64.21	65.85	65.06	66.13	65.36	66.92	49.84	51.68	46.69	46.47	44.64	45.57
Dec. 16	67.89	66.88	69.24	68.25	70.50	66.67	52.09	50.42	53.48	48.73	50.36	47.11
Jan. 12	64.93	68.45	65.68	67.64	67.11	68.28	51.58	52.25	49.05	49.54	46.88	46.36
Feb. 9	65.60	65.83	66.37	67.05	67.98	67.13	50.04	50.58	47.86	49.55	44.59	43.14
Mar. 9	65.44	63.33	65.52	66.28	66.29	66.65	50.86	49.14	49.58	45.92	44.85	45.20
Apr. 6	66.72	67.12	67.33	67.63	67.56	69.32	51.64	50.83	48.96	47.22	46.24	44.88
May 5	67.33	66.42	65.13	66.12	68.44	66.34	49.86	49.41	47.24	46.65	46.01	44.25
June 3	67.43	64.74	67.97	65.50	67.89	66.87	51.71	49.32	47.44	45.93	45.06	44.07
June 20	66.34	64.79	65.54	65.18	65.54	66.92	50.21	49.87	49.28	45.71	45.18	44.85
July 27	67.32	64.91	68.65	65.64	67.04	66.58	49.91	52.03	47.96	50.23	45.92	45.57
Aug. 24	68.43	66.70	69.26	66.50	68.56	66.66	50.26	53.35	52.99	49.58	49.23	48.30
Sept. 28	66.41	64.92	65.66	66.04	66.72	66.06	50.65	49.96	46.36	46.00	46.38	44.25
Oct. 26	66.63	65.49	67.19	66.08	67.12	66.65	50.83	50.69	47.79	47.39	46.79	46.41
Dec. 1	67.38	65.85	66.63	65.95	69.28	67.04	53.38	52.55	49.37	48.50	48.95	47.64

APPENDIX, TABLE II

Seasonal Trend in Alcohol-soluble Material, as a Percentage of Fresh Weight of Bark and Wood at Three Levels

Sampling date.	Bark.						Wood.					
	1 ft.		6 ft.		12 ft.		1 ft.		6 ft.		12 ft.	
	A	B	A	B	A	B	A	B	A	B	A	B
Nov. 10	4.323	4.733	3.639	3.399	3.624	4.006	2.239	1.971	2.395	2.393	2.651	2.387
Dec. 16	3.912	4.525	3.676	3.631	3.374	3.913	2.052	2.207	2.474	2.527	2.846	2.179
Jan. 12	4.343	4.882	4.383	4.302	4.037	4.206	2.439	2.427	2.852	2.587	2.909	2.512
Feb. 9	4.413	5.056	3.845	4.563	4.249	4.099	2.183	2.263	2.616	2.091	2.706	2.244
Mar. 9	4.479	4.115	3.979	3.711	4.028	3.755	2.522	2.251	2.280	1.996	2.087	2.523
Apr. 6	4.503	4.109	4.220	3.638	4.916	3.788	2.081	1.753	2.126	1.689	2.218	2.125
May 5	4.206	4.412	4.149	4.074	4.177	4.057	1.938	1.899	2.086	1.974	2.100	2.220
June 3	4.315	5.045	3.764	4.309	4.275	4.237	2.153	1.948	1.945	2.051	2.034	2.114
June 20	4.359	4.621	4.313	4.213	4.588	4.317	2.011	1.986	2.221	2.060	2.120	2.481
July 27	4.208	4.627	3.838	3.944	3.836	3.846	1.910	1.950	1.874	2.223	1.972	2.225
Aug. 24	3.943	4.137	3.492	4.499	3.391	4.199	1.938	2.494	2.287	2.579	1.822	1.965
Sept. 28	4.158	4.848	3.554	4.118	3.731	3.733	2.187	2.959	2.211	2.141	2.203	2.534
Oct. 26	4.779	5.412	4.212	4.467	4.608	4.720	2.220	2.637	2.364	2.322	2.458	2.497
Dec. 1	4.338	5.531	3.626	4.066	3.629	4.365	1.924	2.142	1.977	2.519	2.409	2.714

APPENDIX, TABLE III

Seasonal Trend in Non-carbohydrate Alcohol-soluble Material, as a Percentage of Fresh Weight of Bark and Wood at Three Levels

Sampling date.	Bark.						Wood.					
	1 ft.		6 ft.		12 ft.		1 ft.		6 ft.		12 ft.	
	A	B	A	B	A	B	A	B	A	B	A	B
Nov. 10	3.661	3.909	2.937	2.380	2.702	2.809	1.596	1.405	1.716	1.754	2.000	1.651
Dec. 16	3.318	3.785	2.893	2.813	2.514	3.027	1.449	1.725	1.569	1.798	2.214	1.691
Jan. 12	3.527	4.146	3.396	3.370	2.913	3.257	1.778	1.823	2.233	1.904	2.284	1.772
Feb. 9	3.790	4.219	3.093	3.549	3.220	3.159	1.654	1.654	1.976	1.435	2.157	1.647
Mar. 9	3.805	3.465	3.167	2.901	3.118	2.894	1.918	1.720	1.760	1.457	1.469	1.937
Apr. 6	3.928	3.533	3.331	2.915	3.060	3.003	1.528	1.240	1.534	1.120	1.641	1.631
May 5	3.608	3.739	3.278	3.157	3.232	3.060	1.446	1.378	1.553	1.411	1.471	1.655
June 3	3.571	4.143	2.926	3.238	3.253	3.240	1.509	1.417	1.386	1.597	1.429	1.546
June 20	3.529	3.815	3.226	3.194	3.277	3.073	1.428	1.403	1.365	1.489	1.318	1.813
July 27	3.686	3.907	3.149	3.079	2.878	2.848	1.418	0.948	1.383	1.574	1.348	1.637
Aug. 24	3.240	3.306	2.641	3.457	2.394	3.132	1.516	1.872	1.826	1.935	1.349	1.328
Sept. 28	3.419	3.949	2.725	2.997	2.872	2.720	1.552	2.060	1.594	1.427	1.590	1.808
Oct. 26	4.263	4.871	3.543	3.745	3.840	3.858	1.677	2.140	1.775	1.747	1.752	1.860
Dec. 1	3.704	4.907	2.926	3.329	2.880	3.461	1.310	1.405	1.397	1.389	1.705	1.867

APPENDIX, TABLE IV

Seasonal Trend in Sucrose, as a Percentage of Fresh Weight of Bark and Wood at Three Levels

Sampling date.	Bark.						Wood.					
	1 ft.		6 ft.		12 ft.		1 ft.		6 ft.		12 ft.	
	A	B	A	B	A	B	A	B	A	B	A	B
Nov. 10	0.522	0.647	0.592	0.855	0.793	0.999	0.471	0.487	0.561	0.563	0.542	0.646
Dec. 16	0.464	0.554	0.629	0.670	0.718	0.683	0.541	0.443	0.754	0.575	0.485	0.415
Jan. 12	0.660	0.601	0.810	0.753	0.925	0.808	0.583	0.405	0.554	0.558	0.523	0.625
Feb. 9	0.485	0.707	0.539	0.865	0.807	0.750	0.418	0.520	0.523	0.571	0.534	0.513
Mar. 9	0.509	0.541	0.657	0.683	0.752	0.715	0.489	0.441	0.425	0.463	0.515	0.484
Apr. 6	0.479	0.468	0.720	0.617	0.740	0.664	0.451	0.426	0.503	0.478	0.488	0.418
May 5	0.481	0.561	0.720	0.707	0.795	0.857	0.410	0.448	0.442	0.488	0.527	0.488
June 3	0.608	0.746	0.690	0.806	0.877	0.817	0.542	0.441	0.479	0.385	0.516	0.470
June 29	0.684	0.661	0.909	0.885	1.158	1.057	0.480	0.490	0.600	0.490	0.644	0.579
July 27	0.417	0.605	0.580	0.733	0.846	0.874	0.428	0.429	0.418	0.579	0.547	0.509
Aug. 24	0.583	0.718	0.734	0.915	0.858	0.924	0.354	0.551	0.398	0.581	0.410	0.558
Sept. 28	0.645	0.790	0.734	0.991	0.732	0.905	0.561	0.795	0.545	0.635	0.533	0.644
Oct. 26	0.420	0.419	0.550	0.579	0.655	0.717	0.461	0.404	0.543	0.491	0.604	0.522
Dec. 1	0.513	0.519	0.564	0.590	0.597	0.727	0.524	0.627	0.507	0.660	0.573	0.722

APPENDIX, TABLE V

Seasonal Trend in Total Reducing Sugars, as a Percentage of Fresh Weight of Bark and Wood at Three Levels

Sampling date.	Bark.						Wood.					
	1 ft.		6 ft.		12 ft.		1 ft.		6 ft.		12 ft.	
	A	B	A	B	A	B	A	B	A	B	A	B
Nov. 10	0.140	0.177	0.110	0.164	0.129	0.198	0.172	0.079	0.118	0.078	0.109	0.090
Dec. 16	0.130	0.186	0.154	0.148	0.142	0.203	0.092	0.120	0.151	0.154	0.147	0.073
Jan. 12	0.147	0.135	0.177	0.179	0.199	0.139	0.078	0.109	0.065	0.125	0.102	0.115
Feb. 9	0.138	0.130	0.213	0.140	0.222	0.190	0.111	0.089	0.117	0.085	0.105	0.084
Mar. 9	0.165	0.109	0.155	0.127	0.158	0.146	0.115	0.081	0.095	0.076	0.103	0.102
Apr. 6	0.156	0.108	0.169	0.106	0.207	0.121	0.102	0.087	0.089	0.091	0.089	0.076
May 5	0.117	0.112	0.142	0.120	0.150	0.140	0.082	0.073	0.091	0.075	0.102	0.077
June 3	0.136	0.156	0.148	0.175	0.145	0.180	0.102	0.090	0.080	0.069	0.089	0.089
June 29	0.146	0.145	0.178	0.134	0.153	0.169	0.094	0.093	0.176	0.081	0.158	0.098
July 27	0.105	0.115	0.109	0.132	0.112	0.124	0.073	0.073	0.073	0.070	0.077	0.079
Aug. 24	0.120	0.113	0.117	0.127	0.139	0.143	0.068	0.071	0.063	0.063	0.063	0.079
Sept. 28	0.094	0.109	0.095	0.130	0.127	0.107	0.074	0.104	0.072	0.079	0.080	0.082
Oct. 26	0.096	0.122	0.119	0.143	0.113	0.145	0.082	0.093	0.106	0.084	0.102	0.115
Dec. 1	0.121	0.105	0.136	0.147	0.152	0.177	0.090	0.110	0.073	0.110	0.131	0.125

APPENDIX, TABLE VI

Seasonal Trend in Starch, as a Percentage of Dry Weight of Bark and Wood at Three Levels

Sampling date.	Bark.						Wood.					
	1 ft.		6 ft.		12 ft.		1 ft.		6 ft.		12 ft.	
	A	B	A	B	A	B	A	B	A	B	A	B
Nov. 10	4.357	3.289	3.781	4.212	3.217	4.093	7.671	8.842	7.361	7.275	4.686	6.769
Dec. 16	4.274	4.459	4.157	5.592	3.394	4.947	8.091	5.805	6.960	5.849	3.973	7.018
Jan. 12	3.339	4.851	3.949	4.851	4.088	5.844	7.607	9.058	6.997	7.133	5.735	7.176
Feb. 9	7.909	6.205	5.996	6.749	6.661	6.712	8.699	7.400	7.611	7.863	7.931	6.576
Mar. 9	4.823	5.373	5.982	8.655	6.874	7.617	7.711	10.217	9.805	10.054	7.650	9.731
Apr. 6	3.002	4.902	3.561	4.511	3.699	5.401	8.142	10.583	6.338	10.285	5.574	7.725
May 5	4.276	4.623	5.699	5.428	5.745	5.677	9.506	7.319	8.355	7.771	6.607	7.080
June 3	5.548	3.932	6.599	5.393	6.964	6.250	8.367	7.967	7.751	8.384	6.943	7.247
June 29	5.650	4.728	5.734	6.024	7.646	6.948	11.007	8.383	8.707	8.510	8.590	7.405
July 27	4.356	3.723	5.038	4.979	7.855	7.022	7.814	9.141	8.271	7.866	8.893	7.436
Aug. 24	4.782	4.744	5.571	6.620	5.577	7.075	6.956	8.820	5.714	10.032	6.478	9.558
Sept. 28	6.337	5.741	6.571	6.620	8.095	7.069	9.313	9.504	7.411	8.794	7.811	7.815
Oct. 26	2.960	3.995	5.135	4.807	4.679	5.821	8.001	9.773	7.052	9.822	6.535	7.532
Dec. 1	2.198	2.267	2.455	2.393	2.173	2.729	6.865	6.291	5.035	4.974	3.606	4.208

Further Experiments on Growth and Fruiting of *Melanospora destruens* Shear. in the Presence of various Carbohydrates, with special Reference to the Effects of Glucose and of Sucrose

BY

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With four Figures in the Text

INTRODUCTION

HAWKER (1939) described the effects of a range of concentrations of a number of carbohydrates on growth and fruiting of *Melanospora destruens* Shear. Mycelial growth was good on glucose media and increased with increase in this sugar up to a relatively high concentration. The number of perithecia produced reached a maximum at a relatively low concentration above which fruiting fell off rapidly. Similar results were obtained with fructose or with a mixture of glucose and fructose, but sucrose produced a different effect. Growth and fruiting were poor with low concentrations of sucrose, but both increased with increase in the amount of sugar up to 10 per cent., when the number of perithecia was greater than with the optimum concentration of glucose. Maltose, lactose, and starch were intermediate in effect, viz. the concentrations of starch or lactose optimal for fruiting were higher than with glucose and less than with sucrose, while the optimum concentration of maltose was the same as with glucose but falling off in fruiting above this was less rapid.

An examination of the relative rates at which glucose and sucrose were used and of the dry weight of the mycelium formed showed that: (i) with low concentration (0.5 per cent.) of sucrose, the medium never contained more than a trace of hexose sugar and a starvation type of growth resulted; (ii) with relatively high concentrations of sucrose the rate of inversion and utilization were such that a concentration of hexoses favourable for fruiting was maintained for some time; (iii) sucrose was removed from the medium more rapidly than glucose, but a greater dry weight of mycelium was formed on a glucose medium than on one with a similar concentration of sucrose, thus indicating an uneconomic use of the latter which it was suggested was probably due to a higher rate of respiration. The rate of respiration has recently been shown to be higher on a sucrose than on a corresponding glucose medium (Hawker, unpublished data).

¹ This work was begun in the Department of Mycology and Plant Pathology, Imperial College of Science and Technology, London.

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The favourable effect on fruiting of a relatively high concentration of sucrose could be reproduced in part, but not entirely, by growing the fungus on a medium originally containing 0.5 per cent. glucose and replenishing the glucose at intervals. Since this attempt to provide a sustained favourable concentration of hexose sugar, similar to that produced by the action of the fungus on a high initial amount of sucrose, was not entirely successful, it was suggested that 'there is an important difference in the manner in which glucose and sucrose are utilized so that the latter is not entirely replaceable by the end products of its inversion' and that 'labile forms of glucose or fructose or both "may" play a significant part in the metabolism'.

Results of a similar kind were obtained by Brown (1925) with *Fusarium fructigenum* and by Hawker and Chaudhuri (1946) with several species of Ascomycetes. These fungi all showed a response to glucose similar to that of *M. destruens* (viz. increase in mycelial growth up to a relatively high concentration where, presumably, osmotic factors become inhibiting, and a rapid falling off in sporulation with increase in amount of sugar above a rather low optimum). Response to more complex carbohydrates varied. With some of these a starvation growth resulted, where presumably the appropriate enzymes were lacking. Others were hydrolysed so rapidly that the effect was similar to that of glucose. More often fruiting was favoured by the more complex substances and it was concluded that the rate of hydrolysis was less rapid.

Maximum fruiting is associated with hydrolysis of di- or polysaccharides which suggests that some labile intermediate product of hydrolysis may be of importance in inducing fruiting. Recent work on carbohydrate synthesis and breakdown by various living organisms emphasizes the importance of phosphorylation. Thus Doudoroff (1943) reported that dry preparations of *Pseudomonas saccharophila* phosphorylated sucrose to glucose-1-phosphate and fructose and conversely if these were provided the formation of sucrose could be shown. Semeniuk (1943) demonstrated the formation of phosphoric esters during the utilization of glucose by *Chaetomium funicola*, and similar results were obtained by Marshall (1942) with *Rhizopus suinus*. The possibility that the readiness with which certain phosphoric esters are formed determines the intensity of fruiting is examined in the present paper.

EXPERIMENTAL METHODS

These were in general similar to those described in previous papers. The salts of medium A (Asthana and Hawker, 1936), viz. KNO_3 , 3.5 gm.; MgSO_4 , 0.75 gm.; KH_2PO_4 , 1.75 gm., were dissolved in a litre of water and a standard dose of growth substances was supplied in the form of 0.2 per cent. (dry weight) of an extract of lentils (Hawker, 1936). This basal medium will be referred to as medium AB. Appropriate amounts of purified preparations of various carbohydrates were added. By means of bioassays with the test organisms *Phycomyces Blakesleeanus* and *Nematospora gossypii* it was demonstrated that none of these preparations contained significant amounts of

aneurin or biotin, both of which stimulate growth and fruiting of *M. destruens* (Hawker, 1939a).

Cultures were incubated at 25° C., unless otherwise stated. Perithecial frequency was estimated by the method of Asthana and Hawker (1936). Estimations of carbohydrate residues were made by Bertrand's methods as described by Plimmer (1915).

EFFECT OF RATE OF BREAKDOWN OF SUCROSE ON GROWTH AND FRUITING

Table V of the previous paper (Hawker, 1939) shows that rapid inversion of sucrose, either by acid hydrolysis or by invertase, gives a result comparable to that with a similar initial amount of glucose, fructose, or a mixture of the two. If, however, the hypothesis that fruiting is stimulated by some factor dependent on the continuous breakdown of sucrose be correct, one might expect that *small* increases in the rate of inversion would further stimulate fruiting.

(a) Addition of invertase to a medium containing sucrose

A preparation of invertase, of which 1 c.c. was stated to reduce the optical rotation of 4 gm. of sucrose to zero in 12 minutes at 20° C., was obtained from B.D.H. Ltd. This was sterilized by passage through a bacterial filter and diluted with sterile water.

Plates of agar medium AB with the addition of 5.0 per cent. sucrose were inoculated at the centre with *M. destruens*. Four days later two pieces of agar 1 cm. square were removed at a distance of 1–2 cm. from the inoculum. The holes thus made were filled with 0.2 c.c. of dilutions of the invertase preparation ranging from 10^{-3} to 10^{-8} . Text-fig. 1 shows a control plate in which the mycelium was thin and spreading and perithecia were formed more or less evenly over the surface with a tendency to earlier and denser production at the periphery. The removal of squares of agar from such a plate had no effect. Figs. 4a–c respectively show the effect of adding invertase solutions of 10^{-8} , 10^{-6} , 10^{-4} times the original strength. The most dilute solution caused the formation of a ring of perithecia round the hole. These formed earlier than those on the rest of the plate. The less dilute solutions gave a zone of vigorous mycelial growth immediately surrounding the holes (resembling that on medium AB+5.0 per cent. glucose) with some stimulation of fruiting at the edge of this zone. These results suggest the more rapid production of some stimulatory substance or substances by the action of the dilute invertase preparation on the sucrose. It is unlikely that this stimulatory effect could be due to the presence of growth substances in the invertase preparation since an adequate amount of these was already present in the medium. Moreover, boiled invertase had no such effect while local increases in the concentration of growth substances (by the application of dilute lentil extract or a solution of aneurin to the holes) did not increase fruiting.

It is also unlikely that the stimulatory effect is due to a transition from the original sucrose medium to a glucose one (produced by the action of the

invertase) since no such stimulation is seen along the line of junction when a segment of a glucose medium is replaced by a sucrose one (Fig. 3) or vice versa.

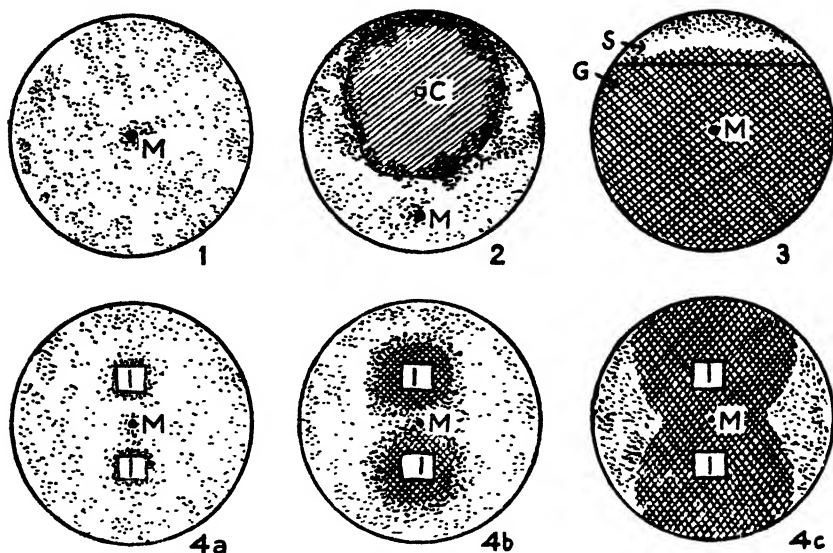


FIG. 1. Culture of *M. destruens* on medium AB + 5.0% sucrose. M = inoculum. Mycelial growth thin.

FIG. 2. Mixed culture of *M. destruens* and *Chaetomium cochliodes* (shaded area) on medium AB + 5.0% sucrose. Note ring of perithecia of *Melanospora* surrounding colony of *Chaetomium*. Perithecia of *Chaetomium* not shown in diagram. c = inoculum of *Chaetomium*.

FIG. 3. Culture of *M. destruens* on medium AB + 5.0% glucose. s = inserted segment of medium AB + 5.0% sucrose. Cross-hatched area = area of vigorous mycelial growth.

FIGS. 4a-c. Cultures of *M. destruens* on medium AB + 5.0% sucrose with dilutions of (a) 10^{-8} , (b) 10^{-6} , and (c) 10^{-4} , respectively, of stock solution of invertase added to holes in agar plate (1). Cross-hatched areas are areas of vigorous mycelial growth characteristic of a glucose medium.

Owing to dense growth of mycelium in some cultures, photographs were unsatisfactory. In these diagrams the black dots represent perithecia. The dots are spaced to give a representation of the relative density and distribution of perithecia but each dot does not represent a single perithecium.

Other experiments in which the distance of the holes from the inoculum or the date of application of the invertase solution were varied gave essentially similar results.

(b) Effect of the presence of other fungi able to break down sucrose rapidly

Certain fungi known to break down sucrose more rapidly than does *M. destruens*, viz. *Chaetomium cochliodes* and *Pyronema confluens* (Hawker and Chaudhuri, 1946) and *Fusarium fructigenum* (Brown, 1925), were grown in mixed culture with the former on agar medium AB + 5.0 per cent. sucrose. Perithecia of *M. destruens* were formed earlier and in greater number in mixed than in pure cultures. A definite ring of perithecia was produced surrounding the colonies of *Chaetomium* (Fig. 2) and *Fusarium*, but the mycelium of

Melanospora intermingled with that of *Pyronema* and produced numerous perithecia over the entire area. In liquid cultures where the medium was kept homogeneous by frequent shaking and where the mycelia were thus more closely intermingled than on agar cultures, perithecia of *M. destruens* were again formed earlier and in greater number in mixed than in pure cultures. Asthana and Hawker (1936) showed that many fungi, including *C. cochliodes* and *F. fructigenum*, stimulated perithecial production by *M. destruens* in mixed culture on standard medium A (i.e. with 0.5 per cent. glucose and without any addition of growth substances) and that this was due to the production of growth substances by the intruder colony. The stimulation on medium AB+5.0 per cent. sucrose, however, is unlikely to be due to the production of growth substances by the intruder colony since an adequate supply is already present in the medium. Moreover, no such stimulatory effect was produced by these fungi on medium A+lentil extract or on medium AB+5.0 per cent. glucose. It is suggested that the stimulatory effect is correlated with a more rapid breakdown of sucrose by the intruder than by *M. destruens* alone. Such a colony, therefore, probably acts in a similar manner to the drops of dilute invertase solution in the previous experiment.

(c) Effect of temperature

Attempts were also made to alter the rate of action of enzymes produced by the fungus and thus of breakdown of sugar by modifying the environment.

Plates of medium AB with 0.5 per cent. glucose, 5.0 per cent. glucose, 0.5 per cent. sucrose, or 5.0 per cent. sucrose were inoculated with *M. destruens* and incubated at a range of temperatures from 15° to 35° C. Radial growth and perithecial frequency were measured. Dry weight of mycelium and rate of removal of sugar from the medium were estimated in a parallel series of liquid cultures which were sampled 2, 3, 6, and 10 days after inoculation. The results are given in Table I and establish the following: (1) on glucose media mycelial growth was greatest at 30° C. (except in samples taken 10 days after inoculation on a medium with a low initial amount of glucose where autolysis had set in at 25° C. and 30° C.). This is in agreement with the results of Asthana and Hawker (1936) on medium A. On sucrose media the optimum was 25° C. (2) The rate of removal of sugar was parallel to the rate of mycelial growth, but the ratio of sugar used to dry weight of mycelium was higher with sucrose, as in the previous paper. This uneconomic use of sucrose is most marked at the higher temperature. (3) No perithecia were formed at any temperature on AB+5.0 per cent. glucose, but the optimum temperature on AB+0.5 per cent. glucose was lower than that for mycelial growth, viz. 25° C. Conversely the optimum temperature for fruiting on the sucrose media was higher than that for mycelial growth, viz. 30° C., while a few perithecia were formed at 35° C. None were formed at 35° C. on AB+0.5 per cent. glucose. At 15° C., however, perithecia were formed earlier on the glucose medium than on the sucrose one. Similar experiments gave comparable results.

TABLE I
The Effect of Temperature on mycelial Growth and Fruiting of M. destruens on Glucose and Sucrose Media

Sugar in original medium.	Age of culture (days).	Dry wt. (mg.) of mycelium per 100 c.c. medium.					Wt. (gm.) of sugar consumed per 100 c.c. medium.					Perithecial frequency.				
		15°C.	20°C.	25°C.	30°C.	35°C.	15°C.	20°C.	25°C.	30°C.	35°C.	15°C.	20°C.	25°C.	30°C.	35°C.
Glucose, 0.5%	3	N.	20	60	63	30	—	—	—	—	—	No perithecia				
"	4	15	55	82	105	71	0.04	0.16	0.21	0.38	0.15	0	F.I.	"	0	0
"	6	71	140	143	157	93	0.22	0.31	0.40	0.50	0.35	0	0.2	0.4	0	0
"	10	140	165	137	125	113	0.47	0.49	0.50	0.50	0.50	0.1	0.8	2.6	0.2	0
"	15	—	—	—	—	—	—	—	—	—	—					
Glucose, 5.0%	3	N.	30	38	47	18	—	—	—	—	—	No perithecia				
"	4	30	50	80	126	60	0.40	0.52	0.64	0.84	0.60	"	"	"	"	"
"	6	38	220	260	278	131	0.88	1.82	2.41	2.45	2.13	"	"	"	"	"
"	10	230	379	393	478	335	1.02	1.80	3.42	3.97	2.50	"	"	"	"	"
"	15	—	—	—	—	—	—	—	—	—	—					
Sucrose, 0.5%	3	N.	11	29	25	18	—	—	—	—	—	No perithecia				
"	4	10	20	25	21	15	0.12	0.29	0.37	0.38	0.15	"	"	"	"	"
"	6	21	31	36	30	26	0.22	0.49	0.49	0.48	0.36	0	0	0	F.I.	0
"	10	30	52	61	55	31	0.33	0.50	0.50	0.50	0.50	0	0	0	0.8	0
"	15	—	—	—	—	—	—	—	—	—	—	0	0.2	0.4	0.9	0.2
Sucrose, 5.0%	3	N.	18	48	37	21	—	—	—	—	—	No perithecia				
"	4	5	43	102	95	50	0.30	2.00	2.93	2.52	1.12	"	"	"	"	"
"	6	50	242	325	283	140	1.16	1.82	3.94	3.39	2.51	0	F.I.	"	M.I.	F.I.
"	10	178	275	278	255	213	3.65	3.19	4.22	5.00	4.03	0	2.0	6.4	8.6	0.4
"	15	—	—	—	—	—	—	—	—	—	—	F.I.	6.6	7.2	10.2	0.4

N. = negligible growth. F.I. = few, immature perithecia. M.I. = many, immature perithecia.

These results admit of explanation along the following lines: (1) *Mycelial growth*. The lower optimum temperature for growth on a sucrose medium may be due to the higher rate of respiration and consequently of removal of sugar from the medium so that sugar concentration becomes limiting at higher temperatures. (2) *Fruiting*. The higher optimum temperature for fruiting on a sucrose medium may be the result of the effect of temperature on the enzymatic breakdown of sucrose. Thus the optimum for fruiting would be the result of the combination of the effect of increase in temperature on rate of enzyme action, on rate of formation of the enzymes, and on rate of their deactivation. The optimum temperature for invertase action is 54° C. (O'Sullivan and Thompson, 1890), but no data are available for the phosphorylating enzymes.

(d) *Effect of hydrogen-ion concentration*

Media AB+0.5 per cent. glucose and AB+5.0 per cent. sucrose were adjusted to give a range from pH 3.5 to 9.6. On both media mycelial growth was good from pH 4.6 to 8.6 with a poorly defined optimum at pH 6.2. (This is a slightly smaller range than that on medium A reported by Asthana and Hawker (1936). This may be due to a loss of acid toleration by the fungus in artificial culture similar to that described by Horowitz et al. (1945) for *Neurospora sitophila*.) Perithecia were formed on the glucose media from pH 4.6 to 8.8 with a poorly defined optimum at pH 6.2 and on the sucrose media from pH 4.1 to 7.6 with an optimum at pH 5.5 (Table II). Thus the

TABLE II

Effect of pH on Fruiting of M. destruens on Glucose and Sucrose Media

Initial pH.	Perithecial frequency after 12 days on AB+0.5% glucose.	Perithecial frequency after 12 days on AB+5.0% sucrose.
2.4	0.0	0.0
3.8	0.0	0.0
4.1	0.0	3.8
4.6	1.2	5.3
5.0	1.7	7.5
5.5	1.8	6.7
6.2	2.0	5.8
7.6	1.9	4.6
8.8	1.5	0.0
9.2	0.0	0.0
9.6	0.0	0.0

optimum initial pH for fruiting was lower on a sucrose than on a glucose medium. All the media became less acid during the experiment but the relative degree of acidity remained the same. It is of interest that Nelson and Bloomfield (1924) showed that the optimal activity of invertase occurs, at all temperatures, between pH 4.5 and 5.0.

In other experiments pieces of agar were removed from 2-day-old cultures on AB+0.5 per cent. glucose and AB+5.0 per cent. sucrose and were

replaced with media of higher acidity. Perithecia were more numerous on and around the inserted piece than in the rest of the plate on the sucrose medium, but fruiting was uninfluenced on the glucose medium.

(e) *Inhibition or deactivation of enzymes by certain substances*

Experiments on the inhibition or deactivation of enzymes on a sucrose medium by the addition of glucose or fructose, which partially inhibit invertase (Kuhn and Münch, 1925), or of certain poisons (Haldane, 1930) were in general inconclusive. Drops of weak solutions of mercuric chloride or silver nitrate placed at the edge of young colonies on medium AB+5.0 per cent. sucrose were later surrounded by a clear zone in which no hyphae grew, which in turn was surrounded by a zone in which hyphae penetrated but no perithecia were formed. No such mycelial zone was formed on medium AB+0.5 per cent. glucose. It is suggested that in this non-fertile zone on the sucrose medium the concentration of the poison was insufficient to prevent growth but sufficient to slow down the hydrolysis of sucrose.

The experiments of this section are in accordance with the view that the rate of hydrolysis of sucrose and the concentration of intermediate products of hydrolysis influence fruiting.

EFFECT OF OTHER SUBSTANCES YIELDING FRUCTOSE ON HYDROLYSIS OR
STRUCTURALLY ALLIED TO FRUCTOSE

It was thought possible that labile forms of fructose produced during hydrolysis of sucrose might contribute to the favourable effect on fruiting of a high initial concentration of sucrose. Accordingly other substances yielding fructose as an end-product of hydrolysis were tested.

(a) *Raffinose*

Equal quantities of glucose, fructose, and galactose are produced when raffinose is completely hydrolysed. Kuhn and Münch (1925) showed that invertase prepared from yeast acted on raffinose to produce fructose and melibiose, while extracts of *Aspergillus niger* and *Penicillium* sp. did not affect raffinose (Kuhn and Grundherr, 1926).

M. destruens was grown on medium AB+5.0 per cent. sucrose and on AB plus sufficient raffinose to give an equivalent amount of fructose. Various combinations of glucose, fructose, and galactose were also tried (Table III).

The effect of raffinose on growth and fruiting was essentially similar to that of sucrose and was not produced by any combination of hexose sugars.

(b) *Inulin*

Inulin hydrolyses to fructose and a comparison with sucrose was thus likely to be of interest. *M. destruens*, however, is unable to hydrolyse inulin and produces a starvation type of growth in the absence of other sources of carbon (Hawker, 1939). Growth on media containing inulin and glucose was good, indicating that inulin had no harmful effect. The fungus was

TABLE III

*Effects of Sucrose and Raffinose on Fruiting of M. destruens**

Initial sugar in medium.	Percentage concentration of each hexose sugar (free or combined).			
	0.25.	0.5.	1.0.	2.5.
Sucrose	0.3	1.1	2.4	4.2
Raffinose	0.1	0.5	2.3	4.6
Sucrose + galactose . .	1.8	1.6	0.0	0.0
Glucose + fructose . .	2.7	1.3	0.0	0.0
Glucose + fructose + galactose	1.6	0.0	0.0	0.0

* The figures in the table are perithecial frequencies calculated by the method of Asthana and Hawker (1936).

therefore grown on media AB+5.0 per cent. inulin and AB+2.5 per cent. inulin in mixed culture with four other organisms known to hydrolyse inulin, viz. *Trichoderma viride*, an unidentified bacterium, a species of *Penicillium* isolated from a rotting Dahlia tuber, and *Pyronema confluens* (Claussen, 1912). *T. viride* showed sufficient antibiotic activity to suppress the growth of *Melanospora*, but the colonies of *Penicillium* sp. and the bacterium were surrounded by a dense ring of perithecia of *M. destruens*, while the mycelia of *Melanospora* and *Pyronema* intermingled and numerous fructifications of both organisms were formed, scattered over the plate. As in the experiments with mixed cultures on sucrose described above, this stimulatory effect was unlikely to be due to production of growth substances by the intruder since an adequate initial dose was supplied. It is concluded that it is again due to the production of some substance or substances during hydrolysis.

(c) *Arabinose*

Arabo-furanose shows considerable structural resemblance to fructo-furanose. It is thus of interest that as shown in the earlier paper (Hawker, 1939) arabinose was intermediate in effect between glucose and sucrose, viz. fruiting reached a maximum at a concentration of 1.0 per cent. arabinose (compared with 0.5 per cent. and 5.0 per cent. for glucose and sucrose, respectively) above which it fell off more slowly than with glucose.

(d) *Mannitol*

Mannitol is produced from fructose by certain bacteria and is related structurally to this sugar but cannot exist in a ring form. Fungi, however, are said to be unable to produce mannitol from fructose although they convert other sugars (Anderson, 1938). Thus if the ring structure of fructo-furanose is of significance in the stimulation of fruiting one would expect mannitol to lack such a stimulatory effect despite its relationship to fructose. Hawker (1939) showed that growth of *M. destruens* on a medium containing mannitol as sole source of carbon was poor but increased with increasing concentration of the alcohol, while the number of perithecia produced was not significantly

influenced by concentration. This suggests that mannitol is not readily used by the fungus. Growth of *M. destruens* on medium AB+5.0 per cent. mannitol was stimulated by the presence of other fungi known to use mannitol (viz. *Aspergillus niger* and *Pyronema confluens*), but the production of perithecia was not stimulated.

EFFECT OF SUBSTANCES HYDROLYSING TO OR RELATED TO GLUCOSE AND OF OTHER HEXOSE OR PENTOSE SUGARS

The results of the preceding section suggest that furanose-fructose may stimulate fruiting of *M. destruens* since sucrose, raffinose, inulin, and arabinose stimulate fruiting while mannitol does not. This would not explain the favourable effects of starch, maltose, and lactose which were intermediate between sucrose and glucose in this respect (Hawker, 1939).

(a) Starch, maltose, and lactose

The results of the previous paper with these carbohydrates were confirmed. No stimulatory effect was observed in mixed cultures of *M. destruens* and certain other fungi on media AB+5.0 per cent. starch, AB+5.0 per cent. maltose, or AB+5.0 per cent. lactose, although good growth of the 'intruder' colonies indicated that the carbohydrate was hydrolysed by these.

(b) Glycogen

Various concentrations of glycogen were added to medium AB. The results which are given in Table IV show that glycogen in relatively high concentration favoured fruiting. The effects resembled those of sucrose but perithecia were more numerous and mycelial growth was greater.

TABLE IV

Effect of Glycogen on Production of Perithecia by *M. destruens*

Medium.	Perithecial frequency 10 days after inoculation.
AB+0.25% glycogen	Few, immature
AB+0.5% "	" "
AB+1.0% "	Numerous, immature
AB+2.5% "	3.7
AB+2.5% " +2.5% glucose	Few, immature
AB+2.5% " +2.5% fructose	Numerous, immature
AB+2.5% sucrose	2.2

(c) Mannose

This gave results similar to those with glucose, viz. perithecia were not formed at concentrations above 0.5 per cent. but mycelial growth increased with sugar concentration.

(d) Xylose

This gave results of the same type.

(e) *Glucose-1-phosphate*

Doudoroff's (1943) demonstration of the importance of glucose-1-phosphate in the breakdown and synthesis of sucrose by a bacterium has been referred to above. Glucose-1-phosphate has also been shown to be of importance in the synthesis and breakdown of starch by higher plants (Hanes, 1940, 1940a, 1940b) and of glycogen by yeast and certain animal tissues (Meyer, 1940). Sucrose, starch, and glucose all favour fruiting of *M. destruens* and it is therefore possible that the favourable factor common to all three substances is the ease of production of glucose-1-phosphate.

A 1 per cent. solution of the potassium salt of the ester was adjusted to pH 6.5 with acetic acid and sterilized, either by autoclaving or by passage through a bacterial filter, and was added to the melted agar medium just before pouring. In some experiments the ester was autoclaved with the other ingredients of the medium in the usual way. Table V gives the results of two such experiments, in the first of which the ester was autoclaved with the medium and in the second the filtered solution was added to the autoclaved medium. Autoclaving reduced the activity of the ester.

TABLE V
Effect of Glucose-1-phosphate on Fruiting of M. destruens

Expt. 1	Medium.	Perithecial frequency 3 days after inoculation.	Perithecial frequency 7 days after inoculation.
	AB+5.0% sucrose	Few, immature	3.2
	AB+5.0% sucrose+0.025% glucose-1-phosphate	2.4	4.0
	AB+0.25% glucose+0.25% fructose	Very few, immature	1.9
	AB+0.25% glucose+0.25% fructose+0.025% glucose-1-phosphate	More numerous, immature	2.8
Expt. 2			
	AB+5.0% sucrose	Few, immature	3.4
	AB+5.0% sucrose+0.025% glucose-1-phosphate	5.0	8.2
	AB+5.0% sucrose+0.05% glucose-1-phosphate	5.4	9.1
	AB+0.25% glucose+0.25% fructose	None	1.4
	AB+0.25% glucose+0.25% fructose+0.025% glucose-1-phosphate	Few, immature	4.8
	AB+0.25% glucose+0.25% fructose+0.05% glucose-1-phosphate	Few, immature	4.9

Perithecia were formed earlier and in greater number in the presence of the ester. The beneficial effect was reduced by autoclaving. Experiments in which the ester was added to liquid cultures at the time of inoculation or 1, 2, 3, or 4 days later showed earlier and more numerous perithecia when the addition was made not more than 3 days after inoculation, but later additions had no effect.

In other experiments holes were cut in the agar in plates poured with medium AB+5.0 per cent. sucrose and these were filled with the sterile solution of glucose-1-phosphate. Perithecia formed earlier and in greater number round these holes than in the rest of the plate.

These results suggest that glucose-1-phosphate plays an important part in the metabolic processes leading to the formation of perithecia.

DISCUSSION

The above results all suggest that the beneficial effects of sucrose on fruiting are due to some factor connected with the hydrolysis or phosphorolysis of this sugar. Further experiments to elucidate the nature of this stimulating factor cannot be undertaken immediately since the strain of *M. destruens* used in this and previous investigations has gradually decreased in fertility since its isolation in 1932 and is now too variable for use in exact experiments. A new isolate is available which it is hoped will be physiologically similar to the original, but it is more convenient to discuss the results obtained with the latter now.

Little work has been published on synthesis or breakdown of sucrose by micro-organisms but evidence is available from the study of the mechanisms in higher plants. Thus some investigators have studied the range of carbohydrates which, when supplied to excised leaves of various plants or to storage tissues, leads to the accumulation of sucrose. Nelson and Auchindross (1933) found sucrose was formed in the potato more readily from glucose or fructose than from starch. Leonard (1939) stated that the provision of glucose, fructose, sucrose, maltose, or lactose led to the accumulation of sucrose in excised leaves of cotton or sorghum. Similarly, Virtanen and Nordlund (1934) reported the synthesis of sucrose from either glucose or fructose by wheat or red clover, and McCready and Hassid (1941) listed glucose, fructose, mannose, and galactose as suitable substrata with excised barley leaves, and concluded that the synthesis of sucrose took place by way of phosphorylation. Thus higher plants can obviously synthesise sucrose from a number of simple carbohydrates which suggests some mechanism by which these substances can be interchanged via a common intermediate. Recent work on phosphorylation suggests that the formation of a phosphoric ester is the first step in the process whatever the original substrate may be.

The most detailed study of the mechanism of sucrose synthesis is that of Hartt (1943, 1943a, 1943b, 1944), who used excised leaves of sugar-cane. By the use of various enzyme preparations or of enzyme inhibitors she showed that sucrose could be synthesized from either glucose or fructose. A mechanism of interconversion was shown to be essential. She suggested that phosphorous compounds were important both in synthesis and interconversion and concluded that glucose or fructose was first phosphorylated in the leaf blade by the agency of the energy-rich bond of adenosine triphosphate. Whichever hexose was the substrate the phosphorylated sugar was thought to be converted first to hexose-monophosphate and then to fructose-1:6-diphos-

phate which combined with glucose to give sucrose-phosphate. Hartt suggested that a phosphate acceptor, probably aneurin or riboflavin, accepted the phosphate leaving free sucrose.

If Hartt's theory of synthesis be correct one might expect the first step in the hydrolysis of sucrose by living leaves or by mycelium to be the phosphorylation of the sugar by the donation of phosphate by such a substance as aneurin-phosphate with the subsequent formation of glucose and fructose-diphosphate. If the formation of this ester be a step in the stimulation of fruiting of *M. destruens* then the favourable effects of compounds such as sucrose, raffinose, or inulin, which contain fructose, or of arabinose, which is related to furanose-fructose, would be partially explained. If Hartt's theory be accepted, it by no means follows that the course of reaction is reversible. Moreover, such a mechanism would not readily explain the favourable effects of glycogen, starch, maltose, or lactose.

The work of Doudoroff et al. (1943) is of particular significance. It was shown that dry preparations of *Pseudomonas saccharophila* phosphorylated sucrose to glucose-1-phosphate and fructose, and that with these as substrata sucrose was synthesized. It is now well known that the production of glucose-1-phosphate is a stage in the synthesis or breakdown of starch and glycogen by certain plant and animal tissues (Hanes, 1940, and Meyer, 1943). If the production of glucose-1-phosphate is the stimulating factor in perithecial formation by *M. destruens*, the favourable effects of starch, glycogen, and sucrose compared with glucose would be expected since the conversion of the latter to glucose-1-phosphate by way of glucose-6-phosphate would probably require a relatively large expenditure of energy. The favourable effects of raffinose and inulin are also not inconsistent with the view that glucose-1-phosphate is the significant substance since this ester may probably be formed from fructose-1:6-diphosphate by way of fructose-6-phosphate and glucose-6-phosphate with a relatively low expenditure of energy. The effect of lactose is obscure since little is known of the part played by phosphorylation in the breakdown of this sugar. Glycogen is favourable to fruiting and it is significant that this carbohydrate is present in the young asci of *M. destruens* and of many other ascomycetous fungi. It may even be possible that ease of synthesis of glycogen which is dependent on the production of glucose-1-phosphate is one of the factors which control fruiting.

If the energy required to form glucose-1-phosphate from hexose sugar is greater than that required to produce it from sucrose and other di- and polysaccharides, previous results in which the stimulatory effect of aneurin was correlated with an increased rate of respiration become of significance. Moreover, the rate of respiration on a sucrose medium is greater than on a glucose one. It may be that the available energy is one of the factors controlling fruiting rather than the actual production of a particular substance such as glucose-1-phosphate or fructose-1:6-diphosphate. Further work on the correlation between respiration-rate and formation of perithecia is in progress.

The results of experiments with invertase are not readily explained. The effects of temperature, pH, inhibitors, &c., might be due to the effect on one or more of the phosphorylating enzymes rather than on invertase. Where the enzyme was added to the holes the results were striking, but again it is not certain that the enzyme preparation contained no other enzyme as an impurity. One might suggest that invertase is necessary for the preliminary splitting of the sucrose molecule before phosphorylation of the fructose could take place. This is unlikely in view of the results of Doudoroff et al. (1943), who stated that the hydrolytic and phosphorolytic properties of their bacterial preparation were in competition. Doudoroff (1943) demonstrated phosphorolysis of sucrose by the action of a preparation of sucrose phosphorylase relatively free of invertase. On the other hand, Leonard (1938) concluded that while invertase was not concerned with the synthesis of sucrose by leaves of sugar-beet or corn it was active in the hydrolysis of the sugar. The presence of invertase in the mycelium of *M. destruens* is readily demonstrated since dried and macerated mycelium is able to invert sucrose. Moreover, the invertase activity of a given dry weight of mycelium increases with repeated sub-culturing on a sucrose medium.

In conclusion it is established that certain di- and polysaccharides are better sources of carbon for fruiting of *M. destruens* than are hexose sugars. While this is partly due to a rate of hydrolysis giving a favourable concentration of hexoses over a relatively long period, the evidence also suggests that the readiness with which certain phosphoric esters, probably glucose-1-phosphate or less probably fructose-1:6-diphosphate, are formed is of significance and that the amount of available energy is also a factor controlling the production of either vigorous mycelium without perithecia or of scanty mycelium with many perithecia.

SUMMARY

Mycelial growth of *M. destruens* increases with increase in concentration of glucose or fructose up to a relatively high value (Ca 20 per cent.) when, presumably, osmotic factors become limiting. Production of perithecia, however, falls off rapidly when the concentration of hexose is increased above 0.5 per cent. Mycelial growth on a sucrose medium increases with concentration but is always less than on a similar glucose medium. Fruiting increases with increase in concentration of sucrose up to a relatively high optimum amount, and the effect of sucrose is thus in contrast to that of glucose.

The local application of dilute solution of invertase to a young culture on a medium containing an initial concentration of 5.0 per cent. sucrose stimulates perithecial production. Strong solutions invert sucrose so rapidly that growth resembles that on a glucose medium.

Production of perithecia on a 5.0 per cent. sucrose medium with an adequate initial concentration of growth substances is also stimulated by the presence of certain fungi known to invert sucrose more rapidly than does *Melanospora*.

Alterations in temperature and pH of the medium in the direction of the optima for invertase activity increase perithecial production on a sucrose medium more readily than on a glucose one. In all these experiments there is no certainty that phosphorylating enzymes are not also active.

Growth and fruiting on media containing a range of concentrations of raffinose resemble those with concentrations of sucrose plus a corresponding content of fructose. Inulin is not used by *M. destruens* but fruiting of the latter on an inulin medium in the presence of adequate growth substances takes place freely in the presence of other micro-organisms able to hydrolyse this carbohydrate. It is further pointed out that the favourable effect of arabinose is of significance in view of the similarity between arabo-furanose and fructo-furanose. On the other hand, mannitol which is structurally related to fructose but has no ring structure does not stimulate fruiting.

Starch, maltose, lactose, and glycogen in relatively high concentration are also more favourable to fruiting than glucose or fructose. The effects of mannose and xylose resemble those of glucose.

The addition of small amounts of glucose-1-phosphate to media containing 5.0 per cent. sucrose or 0.25 per cent. glucose+0.25 per cent. fructose increases the number of perithecia formed.

The significance of these results is discussed and it is concluded that the amount of available energy and the readiness with which certain phosphoric esters are formed are factors controlling the production of vigorous mycelium with few or no perithecia or of less vigorous mycelium with numerous perithecia.

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A Critical Examination of the Gravimetric Method of determining Suction Force

BY

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With three Figures in the Text

INTRODUCTION

A FAMILIAR method of determining suction force is to weigh pieces of a tissue before and after immersion in a series of solutions at different osmotic pressures. It is assumed that the mean suction force is equivalent to the osmotic pressure of the solution in which the tissue does not change weight. Experiments described in the present paper indicate that this assumption is incorrect. It appears that values of suction force determined by this method are too high, owing to a systematic error in the method.

The present investigation arose from an examination made by one of us (E. A.) of a method of measuring suction force used by N. A. Maximov in the Plant Physiology Institute of the Academy of Sciences of the U.S.S.R. The method is to immerse pieces of leaf in solutions at different osmotic pressures, and to find the solution in which there is no change in concentration as measured with a refractometer. It was designed for the routine determination of suction force in the leaves of cotton under irrigation, and it is said to have given consistent and satisfactory results. This new method had not, however, been compared with the gravimetric method. In the experiments to be described the values given by the two methods were compared. The methods were found to be inconsistent, and we present an analysis of the inconsistencies between them.

The plant material used for the experiments was carrot tissue, potato tissue, and Iris leaves. Carrot and potato tissue were prepared for experiment by punching cylinders of uniform tissue with a cork borer 5.12 mm. in diameter, and cutting the cylinders into slices of uniform thickness. These slices were dried between filter-paper under a weight of 50 gm. before being used. Iris leaves were prepared for experiment by punching discs from the tops of the leaves with a cork borer 8 mm. in diameter.

REFRACTOMETER METHOD

Exactly 1 ml. of each of a series of sucrose solutions (1.0 to 0.1 M) and distilled water is put into a series of weighing-bottles (4×1.5 cm.). The bottles are closed with ground-glass stoppers. Drops of solution are with-

drawn from each bottle and their refractive indices determined with an Abbé refractometer. It was found that indices accurate to 0.0002 could be obtained without elaborate measures to control the temperature of the prism. Slices of carrot tissue, 2 mm. thick and prepared as described above, are dropped into each bottle. Preliminary experiments showed that the slices of carrot came into osmotic equilibrium with all but hypertonic solutions in less than



FIG. 1. Change in refractive index of sucrose solutions to which carrot tissue has been added, plotted against molar concentration of sucrose solutions.

2 hours, and that there was no evidence that sucrose entered the cells during this period. When it is certain that equilibrium is established, drops of solution are withdrawn from each specimen tube in turn, and their refractive indices determined again. A typical set of data is reproduced in Fig. 1, where the difference in refractive index caused by immersion of carrot slices in the solutions are plotted against the concentrations of the solutions. Several features in this figure call for comment:

(a) The carrot slices cause no change in refractive index of a 0.3 M solution of sucrose. This indicates that the suction force of the sample of carrot tissue is equivalent to 0.3 M sucrose.

(b) In concentrations above 0.3 M the refractive indices of the solutions decrease, due to the withdrawal of water from carrot tissue into the sucrose solutions.

(c) Between 0.7 and 0.8 M solutions there is a discontinuity in the curve. This discontinuity occurs in most experiments. It corresponds to the occurrence of plasmolysis. At concentrations higher than those which cause plasmolysis (0.8–1.0 M) there is a steep fall in the curve, which corresponds to a marked shrinking of the vacuole and surrounding protoplasm.

(d) It is evident from the shape of the curve that the refractive index is much more sensitive to losses of water from the tissue in hypertonic solutions than it is to gains of water by the tissue in hypotonic solution. This is due to the fact that the refractive index of a solution, although it bears a linear relation to concentration, bears a hyperbolic relation to volume. If n = refractive index, m = mass of sucrose, v = volume of solution, and c = refractive index of water, the relation between these variables is:

$$n = a \frac{m}{v} + c$$

and the change in refractive index is related to change in volume as follows:

$$\frac{\partial n}{\partial v} = - \frac{am}{v^2}.$$

Refractive index is therefore more sensitive to changes in volume at high than at low concentration.

(e) Carrot slices in water cause a slight rise in the refractive index (0.0003 in the experiment illustrated in Fig. 1). This is presumably due to exosmosis or to loss of solute from cut surfaces on the carrot. A change of this magnitude would be produced by the release of 2.13 mg. of sucrose into 1 ml. of water. The effect on refractive index of a change in sugar content at constant volume is given by the expression

$$\frac{\partial n}{\partial m} = \frac{a}{v}.$$

Therefore the effect is constant through the whole range of concentrations. If it is assumed that the carrot slices release the same amount of solute into the solutions at every concentration it means that the solutions are not exactly 0.1, 0.2, 0.3 . . . M, but are all slightly more concentrated. However, the increase in concentration brought about by this cause is of the order of 0.006 M, and for the purpose of the present investigation this error can be safely disregarded. Indeed, some tissues (e.g. Iris leaf) when put into water do not cause any change in its refractive index at all.

GRAVIMETRIC METHOD

The gravimetric method for determining suction force of tissues is so familiar that a description is not necessary here. But it is necessary to draw attention to a condition without which the method does not give consistent results, namely, the need for a constant and light pressure when the tissue is dried between filter-paper before it is weighed. Some workers do not record

that they took any precautions to dry at constant pressure. Others (e.g. Baptiste, 1935; Reinders, 1938) record that the tissues they used were dried

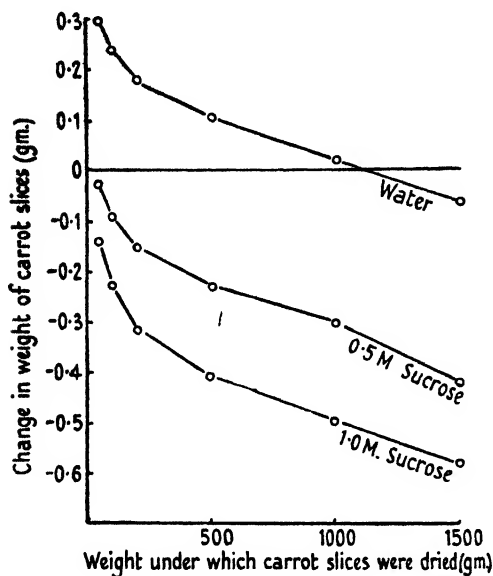


FIG. 2. Change in weight of carrot slices immersed in water, 0.5 M sucrose, and 1.0 M sucrose, and dried between filter-paper under weights of 50, 100, 200, 500, 1,000, and 1,500 gm.

under a constant pressure, but it is not reported that the effect of varying pressure was studied. In order to standardize the gravimetric method for our experiments, we carried out a series of tests in which carrot tissue was immersed in various concentrations of sucrose solution and dried under various weights. The weights were gently laid on the filter-paper under which the carrot slices were lying; care was taken to avoid any shearing pressure. These tests showed beyond doubt that the results of the gravimetric method depend on the weight applied during drying. Some of the data are summarized in Fig. 2.

From these tests the following relevant points emerge: (a) Pressures under weights from

50 to 1,500 gm. for 5 seconds during drying cause the tissue to lose weight; the loss increases with the weight applied. (b) Consistent results are obtained if a relatively light weight (50 gm.) is used for all drying operations in a standardized treatment (5 seconds and without shearing).

COMPARISON OF REFRACTOMETER AND GRAVIMETRIC METHODS

It appears that no doubt has hitherto been thrown on the reliability of the gravimetric method for determining suction force. When one of us (E. A.) made in Moscow a comparison of the gravimetric and refractometer methods, the results were far from identical: values for the suction force of carrot tissue, calculated from the change in weight of discs immersed in a series of sucrose solutions, were invariably higher than values calculated from the change in refractive index of the solutions. The technique adopted for making these comparisons was as follows:

To a series of weighing-bottles were added 1 ml. of solutions of 1.0–0.1 M sugar and water. Sets of freshly cut carrot slices (from secondary phloem), 2 mm. in thickness, were dried between filter-paper under a 50-gm. weight for 5 seconds, and weighed to the nearest 0.1 mg. A set of slices was put into each of the solutions. When osmotic equilibrium had been reached the refractive indices of the solutions were determined, after which the carrot

slices were removed from the weighing-bottles, dried by the same standard technique, and weighed again. In this way the null point in change of weight of tissue and the null point in change of refractive index of solution were determined from the same experimental material. In Fig. 3 are presented the results of some of the experiments. All experiments were carried out in replicate sets, and there was such close agreement between replicates that it is

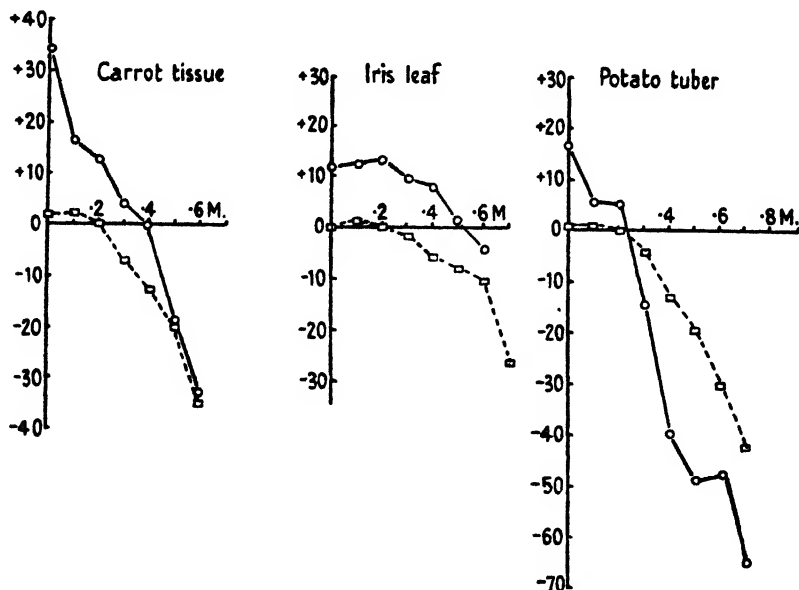


FIG. 3. Values for suction force given by gravimetric method and refractometer method. Abscissae: molar solutions of sucrose. Ordinates: (i) change in weight (mg.) of plant tissue immersed in sucrose solutions (continuous line); (ii) change in refractive index ($\times 10^4$) of external solutions (broken line).

unnecessary to invoke statistical analysis to demonstrate that the two methods give different values.

ANALYSIS OF DIFFERENCES BETWEEN VALUES OF SUCTION FORCE GIVEN BY THE TWO METHODS

For each tissue there is an obvious and consistent difference between the values for suction force given by the two methods. Without exception, differences were large for Iris leaf, somewhat less for carrot tissue, and small for potato tuber. These differences suggest that either the gravimetric method or the refractometer method or both give inaccurate results. Our investigations indicate that it is the gravimetric method which is inaccurate. The inaccuracy is due to the partial injection of the intercellular spaces in some tissues by the solution in which the tissues have been immersed. Therefore the loss of water in hypertonic solutions is partly counterbalanced by the entry of solution into the intercellular spaces. Accordingly the null

point in change of weight of tissue corresponds to a higher osmotic value of the external solution than that equivalent to the suction force; and tissue immersed in a solution isotonic with its suction force shows a gain in weight owing to entry of solution into the intercellular spaces. The refractive index of the external solution is not affected by injection of intercellular spaces, for the solution as a whole, and not only water, enters these spaces.

The evidence for these statements was obtained from the three experiments which follow.

(i) *Injection of intercellular spaces in carrot tissue.* In order to discover whether the intercellular spaces in carrot tissue do become injected with solution during experiments to determine suction force, known volumes of carrot tissue, in sucrose solutions approximately isotonic with the suction force of the carrot (0.2 M), were put into specific-gravity bottles. The bottles were filled as for the determination of specific gravity, and immediately weighed. After 2 hours the volumes of solution in the bottles had decreased, and large air bubbles had appeared under the stoppers. The bottles were again filled with sucrose solutions and weighed. All except the controls showed increases in weight. These increases measure the amount of solution which has entered the intercellular spaces. The experiment was carried out in a constant-temperature room (range of variation in temperature 1° C.); the balance, sugar solutions, bottles, &c., were put into the room 24 hours before the experiment. The experiment was repeated in the same way on pieces of Iris leaf and on slices of potato tissue. A summary of the data is given in Table I.

TABLE I

Percentage of Solution (ml. per vol.) entering Intercellular Spaces of Various Tissues. The Density of Sucrose Solutions used may be taken as Unity within the Limits of Error of the Experiment

	Thickness (mm.).	ml. injected per vol. (%).	S.D.
Carrot (sec. phloem)	2.00	3.233	0.025
" " "	5.00	2.961	0.355
Iris leaf	0.56	4.620	0.544
Potato tuber	2.00	0.744	0.180

These values were obtained from several different samples of material, and their errors therefore include some variation due to material. It appears that in the course of a gravimetric determination of suction force about 3.1 per cent. by volume of carrot tissue becomes injected, irrespective of the thickness of the slices. This represents about a quarter of the total percentage of intercellular space. Slices of Iris leaf immersed in sucrose solutions approximately isotonic with their suction force absorb into their intercellular spaces about 4.6 per cent. by volume of sucrose solution; and slices of potato tuber absorb only about 0.7 per cent. by volume of sucrose solution.

(ii) *Effect of applying corrections for injection to suction force determinations by the gravimetric method.* If the different values for suction force of carrot

tissue from the two methods under discussion are due to the entry of solution into about 3.1 per cent. of the intercellular spaces, then data obtained by the gravimetric method are too high and may be corrected by subtracting a weight of water equal to 3.1 per cent. of the volume of tissue, from all the differences in weight. After this correction has been applied the two methods should give approximately the same results; and, with appropriate corrections, the same should apply to the data on Iris leaf and potato tissue. To test this assumption the suction force of the three tissues was determined simultaneously by the two methods, and corrections applied on the assumption that a known proportion of the volume of tissue was injected during the experiment. The results are summarized in Table II.

TABLE II

Sucrose Molar Equivalents of Suction Force from Refractometer Method (S.F.R.), and from Gravimetric Method (S.F.G.)

Volume (ml.).	Thickness (mm.).	S.F.R.	S.D.	S.F.G.	S.D.	Diff. (1).	S.F.G. (Diff. 2).	
Carrot:								
0.412	1	0.24	0.022	0.39	0.021	0.15	0.28	0.04
0.412	5	0.23	0.023	0.39	0.039	0.16	0.29	0.06
0.206	1	0.24	0.033	0.36	0.022	0.12	0.24	0.00
Iris leaf:								
0.297	0.56	0.20	0.008	0.53	0.011	0.33	0.21	0.01
Potato:								
0.206	2	0.20	0.011	0.24	0.009	0.04	0.22	0.02

The column Diff. (1) contains the differences between values for suction force determined by the two methods. Except for potato, the differences are clearly significant. Column Diff. (2) contains the differences after a correction for injection of intercellular spaces has been applied to values from the gravimetric method. The differences are no longer significant. It is evident that the error in values for suction force as determined by the gravimetric method depends on the volume of the intercellular space system and the degree to which it becomes injected with solution. The error is large in Iris leaves, which have a large volume of intercellular space, and it is negligible in potato, which has a small volume of intercellular space (see Fig. 3). The light drying pressures commonly applied to tissues in the gravimetric method are insufficient to squeeze out solution which has entered the intercellular spaces, though rough tests indicate that under a weight of 1,000 gm. during drying, most of the solution in intercellular spaces is squeezed out.

(iii) *Treatment of tissue to eliminate difference between the two methods.* The assumption that the values of suction force given by the gravimetric method are inaccurate owing to the injection of intercellular spaces is supported by a third series of experiments. In these experiments carrot tissue was equilibrated to a known suction force by immersion in a sucrose solution of known concentration; then its suction force was determined simultaneously by the two

methods. During the process of equilibration the intercellular spaces became injected; and since they were already injected before the first weighing in the gravimetric determination of suction force it followed that the error due to injection was eliminated. Under these conditions both methods gave the same values for suction force, namely, values equal to that of the osmotic pressure of the solution in which the tissue was equilibrated.

SUMMARY

The suction force of samples of carrot tissue, potato tuber, and Iris leaf was determined simultaneously by two methods: (i) the familiar gravimetric method, and (ii) by measuring the change in refractive index of sucrose solutions containing samples of tissue (Fig. 1). Except with potato tissue the two methods gave widely different values: values from the gravimetric method were always considerably higher than values from the refractometer method (Fig. 3).

The cause of the disagreement between the results from the two methods lies in a systematic error in the gravimetric method. While immersed in solutions of different osmotic strengths intercellular spaces of the tissues become partly injected with solution. The solution in the intercellular spaces is not removed when the tissues are dried before being weighed. Hence in a solution isotonic with the suction force of the tissue there is a considerable gain in weight due to the solution in the spaces.

The quantities of solution which enter the intercellular spaces were determined (Table I). When allowance is made for these quantities, by applying a correction to the gravimetric method, the two methods give approximately the same values for suction force (Table II).

In the gravimetric method the pressure applied to tissue as it is dried has a very pronounced effect on the wet weight, and therefore on the values obtained for suction force (Fig. 2).

The senior author began this work as a guest in the Timiryazev Institute for Plant Physiology of the Academy of Sciences of the U.S.S.R. He wishes to record his gratitude to Academician N. A. Maximov, for his courtesy in allowing the author to work in the laboratory, and for initiating him into the use of the refractometer for measuring suction force.

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Studies in the Vernalisation of Cereals

X. The Effect of Depletion of Carbohydrates on the Growth and Vernalisation Response of Excised Embryos

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With two Figures in the Text

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INTRODUCTION

THE response of excised embryos of winter rye to vernalisation has been shown to vary with the concentration of sugar in the medium on which they are germinated (Purvis, 1944). In the entire absence of added sugar, however, a few embryos responded to vernalisation treatment while the majority remained unvernalsed. This behaviour, attributed to stored carbohydrate in the embryo, marred the precision of the experimental data obtained. To eliminate this source of error in later work the embryos were freed from residual carbohydrates by respiration during a period of starvation. In this paper the effect of such treatment is examined and its application to experiments on the role of sugar in vernalisation is discussed.

METHODS

The technique of growing excised embryos on agar medium with or without sugar has been described (Purvis, 1944) and remained unchanged in the experiments now under review. The residual carbohydrate was removed by growing the embryos on sugar-free medium at 20° C. until growth was seen

to have ceased. This 'residual growth' varied somewhat in extent and was considerable, but ceased within 5 days (see Tables I and II, p. 271).

The possible utilization of agar as a carbohydrate was recognized, but a trial showed that the 'residual growth' on an agar medium was no greater than that of embryos supported on glass-wool saturated with a liquid medium. These preliminary tests led to the adoption of a standard 'residual growth' treatment of 4 days on sugarless agar medium at 20° C. At the end of the 'residual growth' period the embryos were transferred with aseptic precautions to the medium on which they were to be vernalised; unless otherwise stated this contained 2 per cent. sucrose. Although individual power of survival is remarkable, 'residual growth' treatment resulted in the death of a number of embryos, and those which survived were flaccid until growth was again established. After this treatment there was an increased susceptibility to fungal contamination of the cultures. As the sugar medium itself is inadequate for the growth of many fungi, it may be that exudations from the flaccid embryos supplied accessory food factors. This exudation must be considered as a possible cause of loss in all embryos so treated, whether contaminated or not.

The seedlings were planted in sand-culture pots during the first week in May. The period following planting was even more critical for starved embryos than for those normally vernalised (cf. Purvis, 1944) and a number failed to produce chlorophyll. The rather serious reduction in replication seen in the tables is due in greater degree to this failure after planting, for which allowance had not been made, than to death during treatment. Sugar feeding after planting facilitated the transition to autotrophism.

Data on embryos' growth were obtained by measuring tracings of the embryos made immediately before and after vernalisation treatment. The flowering behaviour of the plants was determined by a 'scoring' method (Gregory and Purvis, 1938). At the termination of the experiment, plants which had not flowered were examined, and a 'score' less than 50, based on the observed condition of the stem apex, was assigned to each plant. This score approximates the number of days required by spring rye in optimal conditions to reach the stage denoted. On this basis the inception of 'double ridges' (Purvis, 1934) was scored as 21 and anthesis occurring on the day of examination was scored as 49. Values between these were assigned according to the stage reached. In order to give individual plants which had already flowered values comparable with these, the number of days that had elapsed since they flowered was added to 49. Thus the score is a positive measure of earliness, and the implication of a particular score, x , is that on the day on which the plants were examined (say, 130 days after planting) the plant had advanced as far towards flowering as a spring rye plant would have done in x days. The time unit is thus 1 day. From the figures a mean value was obtained denoting the earliness of each series. It should be noted that these mean values are only comparable when determined at the same time after planting.

RESULTS

1. *Effect of the duration of 'residual growth' treatment on the subsequent growth of the embryos and of resulting plants*

The recovery of the embryos after 'residual growth' treatment as shown by their linear growth on medium containing sugar is presented in Tables I and II. Clearly the ability of the embryos to utilize an adequate sugar-supply is progressively lost during the preliminary period of starvation, and this effect is intensified when the sugar is presented at low temperature, i.e. during vernalisation (Table II). Thus, for example, 21 days' starvation at 20° C. reduces subsequent root growth on 2 per cent. sugar to $\frac{1}{5}$ when the temperature remains the same and to $\frac{1}{50}$ when the temperature during the sugar period is 1° C. In 1940 growth on sugar medium without previous treatment was less than in other years, while that in 1941 agreed well with similar measurements in 1939 and 1942.

TABLE I

Embryo Growth (mm.) during 4 Days on Sucrose (2 per cent.) at 20° C. after Different Preliminary Periods of Starvation. (Number of replicates shown in brackets)

Days of 'residual growth' treatment.	Extent of 'residual growth'.		Growth during subsequent 4 days on sugar at 20° C.	
	Coleoptiles.	Roots.	Coleoptiles.	Roots.
0 (10)	—	—	18.0 ± 1.4	47.8 ± 9.8
4 (11)	6.5 ± 0.4	6.9 ± 0.7	5.4 ± 0.7	45.5 ± 9.6
7 (10)	7.0 ± 0.7	7.2 ± 0.7	2.4 ± 0.6	31.8 ± 8.4
14 (6)	7.2 ± 0.8	6.7 ± 0.9	0.7 ± 0.4	17.6 ± 3.4
21 (7)	6.7 ± 0.7	5.8 ± 1.1	0.3 ± 0.3	11.4 ± 2.8
28 (4)	5.7 ± 0.5	7.0 ± 0.8	0.3 ± 0.2	4.4 ± 1.8

TABLE II

Embryo Growth (mm.) during 6 Weeks on Sucrose (2 per cent.) at 1° C. after Different Preliminary Periods of Starvation. (Number of replicates shown in brackets)

Days of 'residual growth' treatment.	Extent of 'residual growth'.		Growth during subsequent 6 weeks on sugar at 1° C.	
	Coleoptiles.	Roots.	Coleoptiles.	Roots.
<i>Experiment of 1940</i>				
0 (10)	—	—	8.9 ± 0.5	16.0 ± 2.1
4 (14)	6.1 ± 0.5	4.9 ± 0.6	1.8 ± 0.4	14.4 ± 2.4
10 (7)	6.7 ± 0.7	7.2 ± 0.8	—0.4	6.9 ± 2.6
<i>Experiment of 1941</i>				
0 (20)	—	—	16.1 ± 2.0	50.2 ± 5.8
4 (19)	7.4 ± 0.4	3.2 ± 0.3	5.7 ± 0.8	14.5 ± 2.1
7 (16)	7.2 ± 0.6	2.5 ± 0.4	0.4 ± 0.3	12.3 ± 2.8
10 (15)	9.2 ± 0.5	3.1 ± 0.3	—1.2 ± 0.3	6.2 ± 1.6
14 (13)	7.7 ± 0.5	2.5 ± 0.4	—0.9 ± 0.3	6.0 ± 1.2
21 (6)	7.3 ± 0.6	3.4 ± 0.5	—0.1 ± 0.2	0.9 ± 0.2

The negative values entered in Table II for coleoptile extension after the starvation period are due to loss of turgor and shrinkage after the coleoptiles

had attained their final length and the first leaf had emerged. It appears, then, that 'residual growth' treatment reduces the final size of the coleoptile as well as its growth-rate, thus accentuating the shortening which always accompanies vernalisation treatment (Hatcher, unpublished data).

The cause of this reduced growth-rate on sugar, which follows 'residual growth' treatment and increases as this is prolonged, cannot lie in the depletion of sugar reserves since sucrose is present in the medium in excess. Either the embryos can no longer absorb sugar after the period of starvation or some other factor requisite for growth is lost during this period.

There is no evidence that the 'residual growth' treatment has any permanent effect on the growth-rate of the plants after planting in sand, but as the experiments were not designed to give data on yield this point remains unsettled. The number of tillers per plant at the conclusions of the two experiments is shown in Table III to be independent of the duration of the 'residual growth'

TABLE III

Effect of 'Residual Growth' Treatment on Number of Tillers per Plant. (Number of replicates shown in brackets)

Days of 'residual growth' treatment.	Tillers per unvernalsed plant after 95 days (1940).	Tillers per vernalised plant after 142 days (1941).
0	21.4 ± 2.5 (10)	5.2 (20)
4	19.6 ± 1.8 (11)	5.3 (19)
7	23.7 ± 6.4 (10)	9.9 (16)
10	—	4.0 (15)
14	21.9 ± 6.6 (6)	4.7 (13)
21	25.4 ± 7.7 (7)	3.0 (1 only)
28	20.7 ± 3.5 (4)	—

treatment. In 1940 'residual growth' treatment reduced the length of the culms of those plants which eared, but this effect was not evident in 1941.

The first leaf is shortened by this treatment in both vernalised and unvernalsed seedlings, the effect increasing with duration of the starvation period, and in vernalised seedlings this accentuates the reduction in leaf length caused by the low-temperature treatment (Thimann and Lane, 1938). This resembles the effect on coleoptiles mentioned above. Leaf lengths are given in Table IV.

TABLE IV

Length of First Leaf (cm.) as influenced by 'Residual Growth' Treatment (1940) (Number of replicates shown in brackets)

Days of 'residual growth' treatment.	Unvernalsed plants.	Vernalised plants.
0	3.3 ± 0.3 (10)	2.4 ± 0.3 (10)
4	3.6 ± 0.2 (11)	2.7 ± 0.2 (14)
7	2.3 ± 0.3 (9)	—
10	—	0.9 ± 0.3 (7)
14	1.2 ± 0.3 (7)	—
21	1.0 ± 0.2 (7)	—
28	0.6 ± 0.2 (4)	—

2. Effect of the duration of 'residual growth' treatment on response to vernalisation

'Residual growth' treatment, without subsequent vernalisation, showed no consistent effect upon the condition of the stem apex of winter rye examined 14 weeks after planting. With spring rye, on the other hand, 7 days' starvation retarded flowering by nearly 1 week over and above the retardation caused by separation from the endosperm (Table 5). Spring rye embryos failed to survive periods of starvation longer than 7 days. The modifying effect of

TABLE V
Effect of Excision and of 'Residual Growth' on Flowering of Spring Rye

	Whole grain.	Excised embryos germinated on 2% sugar.	Excised embryos germinated 7 days without sugar then on 2% sugar.
Number of replicates	15	14	7
Days to anthesis	60.1 ± 0.6	65.9 ± 1.0	72.6 ± 1.7
Difference Due to		5.8 ± 1.2 excision	6.7 ± 1.9 residual growth

different periods of 'residual growth' treatment on the response of winter rye to 6 weeks' vernalisation is given in Table VI. In 1940 when vernalisation was preceded by starvation of either 4 or 10 days' duration, flowering was significantly retarded; though the retardation appeared to be progressive, the various durations were not significantly different in effect. The three plants which received 28 days' preliminary starvation were the survivors of a group

TABLE VI
Mean 'Scores' of Plants grown from Excised Embryos vernalised 6 Weeks on Sucrose (2 per cent.) after Different Periods of 'Residual Growth' Treatment (Number of replicates shown in brackets)

Days of 'residual growth' treatment.	Score at 95 days in 1940.	At 142 days in 1941.
0	70 ± 2.4 (10)	118 ± 2.0 (20)
4	53 ± 6.7 (14)	101 ± 5.6 (19)
7	—	93 ± 8.5 (16)
10	37 ± 8.1 (7)	101 ± 6.0 (15)
14	—	110 ± 3.7 (13)
21	—	109 ± 0.0 (1 only)
28	63 ± 7.5 (3)	—

of 50 embryos used in a preliminary test of tolerance to the treatment. For this reason they do not constitute a random sample but are a group selected because of their survival, and thus cannot be regarded as representative. This consideration, of course, applies to some extent to any series in which there are losses due to the treatment used. These three plants flowered and their mean score is not significantly less than that of the control series. In 1941 none of the plants survived 'residual growth' treatment for 28 days, and only

one reached maturity after 21 days' starvation. Thus reliable data are available for periods up to 14 days only. All these 'residual growth' treatments retarded flowering significantly, but the scores obtained after such treatments did not differ significantly among themselves. It thus remains in doubt whether prolonged treatment results in a progressive retardation followed by a slow reversal of the effect or if the retardation attains a constant value in 4 to 7 days.

3. Use of the 'residual growth' treatment in the study of the relation of sugar concentration to vernalisation response

It has been mentioned that the 'residual growth' technique was primarily designed to determine with more precision the effect on excised embryos of low concentrations of sucrose in the medium during vernalisation treatment. In 1940 excised embryos were given 4 days' 'residual growth' treatment and then vernalised on media containing sucrose in different concentrations. The resultant flowering behaviour is set out in Table VII, which includes for comparison 'scores' obtained without 'residual growth' treatment. The latter repeat data given in Table VI of an earlier paper (Purvis, 1944, p. 296), but there the results were given not as 'scores' but in days to anthesis, and the behaviour of the non-flowering plants assessed in a different manner: the values entered are therefore different.

TABLE VII

'Scores', 95 Days after Planting, of Plants grown from Embryos vernalised for 6 Weeks on Media with various Concentrations of Sucrose. (Number of replicates shown in brackets)

Sucrose (%)	No 'residual growth' treatment. Score.	4 days' 'residual growth' treatment.		
		Score.	Difference from effect of Nil.	
			2 ⁰ 0.	
2	70±2.4 (10)	53±6.7 (14)	—	32±10.5*
0.5	—	50±5.8 (8)	3±9.2	29±7.1*
0.1	—	22±7.3 (3)	31±15.0	1±6.2
0.05	—	36±7.7 (4)	17±13.3	15±7.0
0.01	48±6.4 (10)	19±3.8 (4)	34±12.5*	2±4.5
0.005	—	24±4.7 (4)	29±13.0*	—3±5.0
nil.	37±6.1 (9)	21±2.7 (6)	32±10.5*	—
Unvernalised control	18±4.6 (10)			

*Significant differences ($P = 0.05$).

The sporadic flowering which followed vernalisation for 6 weeks without sugar and without 'residual growth' is reflected in the 'score' at 95 days which exceeds that of unvernalsed winter rye by 19 ± 6.0 , a highly significant difference. Following 4 days' 'residual growth' the same vernalisation treatment results in the insignificant acceleration of 3 ± 2.0 scoring units compared with the unvernalsed control. Thus the suggestion receives support that the slight acceleration which in earlier experiments followed upon low-tempera-

ture treatment without sugar was in fact due to stored carbohydrates in the embryo. In this case the premature termination of the experiment must be considered: continuation of the experiment might have revealed larger differences between the treated series and the unvernalsed controls (cf. Table X). The standard error of the sets vernalised with little sugar is notably reduced by 'residual growth' treatment, but with high concentrations of sugar variance is increased by this treatment presumably owing to the varying degree of retardation of flowering ascribable to the preliminary starvation. The low replication, resulting from the lethal effects of the starvation treatment, was more pronounced when the concentration of sugar during vernalisation was low, and this reduces the precision of the data presented in Table VII. Though these make it clear that the response to vernalisation diminishes with falling sugar concentration, they give no precise indication of a critical concentration level. The accelerating effect of sugar present during vernalisation in concentration less than 0.1 per cent. is not significant, but, on the other hand, the retardation which results from reducing the concentration from 2 per cent. to 0.05 per cent. is again not significant. There remains, however, no doubt that the amount of sugar required for vernalisation of excised embryos is not large.

4. *Transference experiments*

In 1939 embryos were vernalised for a total of 6 weeks with sugar supplied during part of the treatment only, from 1 to 5 weeks on sugar either preceding or following a period without sugar (Purvis, 1944). This experiment was repeated in a modified form in 1941 both with and without 'residual growth' treatment. In this experiment, however, the total vernalisation was extended to 8 weeks to allow time for the process to reach completion. Transfers were made after 2, 4, and 6 weeks of vernalisation treatment from sugarless (nil) medium to 2 per cent. sugar (A) and from sugar to 'nil' (B). Plants which had not flowered at 140 days after planting were assigned 'scores' after dissection as described on p. 270. The results are presented in Table VIII and Fig. 1. Although the absolute values are not the same as in 1939, due in part to climatic differences between the two years, and in part to the extended period of vernalisation used, the general trend of each series remains unchanged; and this is true also when residual carbohydrate has been removed by starvation. The main conclusions drawn in the earlier paper (Table VIII, 1944) are here re-examined to test agreement with the new data in Table VIII.

1. The effect of sugar is again greater when it is supplied before the period on 'nil' medium, but in agreement with hypothesis the removal of residual carbohydrate by the preliminary starvation of the embryo substantially increases the effect of priority of sugar application (row 3).

2. A minimum of 2 to 4 weeks' vernalisation on sugar (combined effect of sugar and low temperature) is required for any significant accelerating effect to be shown when no further low-temperature treatment is given (curve C, Fig. 1).

TABLE VIII

Effect on Flowering Behaviour ('score' 140 days after Planting) of Transference during Vernalisation (A) from Sugarless Medium to 2 per cent. Sucrose; (B) from Sucrose to No-sugar

	None				4 days 'residual growth'			
	0	2	4	6	0	2	4	6
Pre-vernalisation treatment								
Weeks vernalisation with sugar	8	0	4	6	8	0	4	6
Weeks vernalisation without sugar						6	4	2
1. (A) No-sugar to sugar								
2. (B) Sugar to no-sugar								
3. Difference (effect of sugar priority)								
4. On sugar for stated time without further low-temperature treatment								
Accelerating effect of low temperature during no-sugar period.								
5. (A) at beginning (row 1 to row 4)								
6. (B) at end (row 2 to row 4)								
7. (A) per week								
8. (B) per week								
Accelerating effect of sugar during low-temperature period.								
9. (A) at end (in row 1)								
10. (B) at beginning (in row 2)								
11. (A) per week								
12. (B) per week								

3. Even when no sugar is added, 8 weeks' vernalisation gives an acceleration of 57 days. (In 1939, 6 weeks of chilling without sugar accelerated flowering by 44 days.) This acceleration is partly due to residual sugar in the embryo, but clearly not entirely so, since 'residual growth' treatment does not eliminate the acceleration but reduces it only to 31 days.

4. When sugar is given first, though the maximum effect is not reached in 2 weeks as in the experiment in 1939, there is no doubt that within 4 weeks

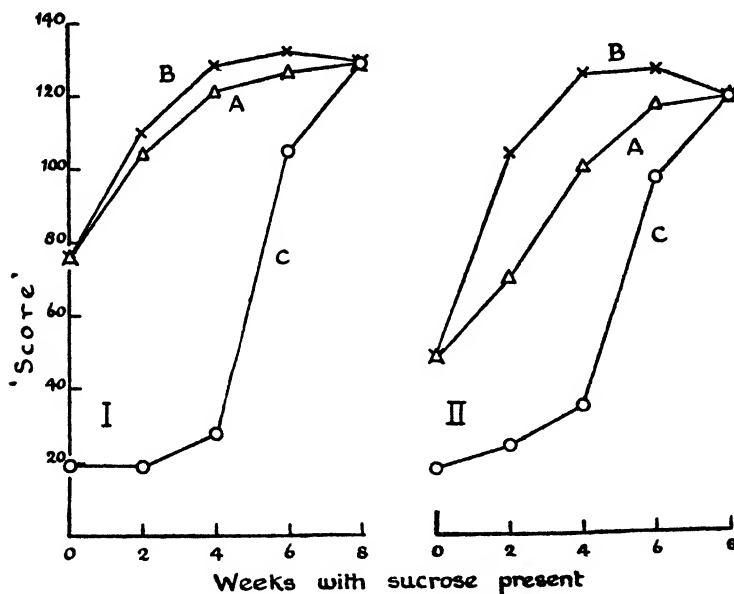


FIG. 1. Excised embryos of Petkus winter rye. Effect on flowering behaviour of supplementing the period of vernalisation on 2 per cent. sucrose medium with a period on 'nil' medium, the total duration of vernalisation being constant at 8 weeks. Curve A, period on 'nil' precedes that on sugar; curve B, 'nil' period follows that on sugar; curve C, no 'nil' period given. *Ordinates*: mean 'scores' in conventional units. *Abscissae*: duration of low-temperature treatment with sugar present. I. No 'residual growth' treatment given. II. 4 days' 'residual growth' treatment precedes vernalisation.

the critical level has been reached, and that the continued presence of sugar in the medium is without further effect. The actual effect of the period on 'nil' following that on sugar is given in row 6 of Table VIII and the effect per week in row 8. This effect increases with the duration of the previous sugar treatment, during which time storage of sugar goes on; this is evidently complete at the end of 4 weeks, after which the effectiveness of the 'nil' period drops suddenly. This drop is not due to any fall in the potential efficacy of the 'nil' period after sugar, but rather to the fact that acceleration associated with rising internal sugar level approaches a maximal value, namely, that found in spring rye, which is unaffected by low-temperature treatment. Were the rate on 'nil', apparent at 4 weeks, i.e. 25 days acceleration per week of treatment, maintained after 6 weeks on sugar, the score would become $115 + 50$ or

165, which is 25 more than that of spring rye at the same age. 'Residual growth' treatment has made no significant difference to the effect of the 'nil' period following sugar treatment; no doubt the amount of 'residual' sugar is too small compared with that absorbed during treatment and the sugar status of the embryo is approximately identical in the two cases.

5. The effect of a nil period given *before* sugar treatment was obscured in the experiment in 1939 by the residual sugar in the embryo. In the work now under discussion, in the presence of this carbohydrate supply, as shown in row 5 of Table VIII, a very considerable acceleration results from this preliminary period on 'nil' medium reaching a broad maximum between 2 and 4 weeks. But even after the removal of residual carbohydrate, 4 weeks on 'nil' medium increases the effect of 4 subsequent weeks on sugar by 66 days. Taken in conjunction with the fact that, after 'residual growth' treatment, 8 weeks' vernalisation on 'nil' medium accelerates flowering by 31 days (see Table VIII), it appears that the accelerating effect of cold is not solely dependent upon added sugar. The effect of 'residual growth' treatment is not the same for all variants. The retardation caused by the preliminary period of starvation for each sugar regime during vernalisation is given in Table IX. The values marked with asterisks are statistically significant.

TABLE IX

Retardation (in Scoring Units) due to 'Residual Growth' Treatment for 4 Days preceding Vernalisation Treatments in Table VIII

Weeks on sugar	0	2	4	6	8
No further treatment	1	-5	-7	17	9*
Sugar before no-sugar	27	6	2	5*	9*
No-sugar before sugar	27	34*	20*	8*	9*

* Significant values ($P = 0.05$).

In conformity with the hypothesis that the main effect of the preliminary starvation period is due to removal of sugar, the accelerating effect of priority of sugar application is accentuated after 'residual growth' treatment.

5. Effect of prolonged low-temperature treatment in the absence of sugar

In the transference experiment described above, treatment of excised embryos for 8 weeks without sugar resulted in slightly accelerated flowering even when the vernalisation period was preceded by 4 days' 'residual growth' treatment. It appeared desirable therefore to investigate the effects of prolonged periods of vernalisation without added sugar, after removing stored carbohydrates from the embryos by 'residual growth' treatment. Few embryos so treated, however, survived the prolonged starvation and of these, in the main, only those which received no preliminary starvation treatment grew to maturity. The results are entered in Table X.

The progressive increase in the score of the plants vernalised for periods up to 12 weeks without added sugar treatment is very striking. The score

TABLE X

'Scores' of 191-day-old Plants, from Embryos vernalised for various Periods at 1° C. without added Sugar. (Numbers of replicates shown in brackets)

Weeks of treatment.	No 'residual growth' treatment.	7 days 'residual growth' treatment.
6	112 ± 14.8 (11)	Dead
8	134 ± 12.4 (14)	139 (3)
12	162 ± 2.0 (12)	Dead
16	155 ± 2.3 (5)	Dead

after 12 weeks' vernalisation is equivalent to an average of 78 days from planting to anthesis, which is little longer than is characteristic of plants vernalised on sugar for 6 weeks. No further increase in score follows prolongation of treatment to 16 weeks; indeed the decline during the final 4 weeks falls just short of significance. The very prolonged starvation may here introduce a complicating factor, since the results up to 12 weeks' exposure suggest that, given sufficient time, vernalisation might be completed in the absence of sugar. These embryos grew very little during the prolonged exposure to cold and starvation, indeed much less than the total amount possible without sugar at room temperature (cf. Purvis, 1944, p. 312); but growth was renewed in all cases on transfer to sugar, and the extent of this growth made during three days at 18° C. is given in Table XI.

TABLE XI

Growth (mm.) of Embryos vernalised without Sugar

Weeks of treatment.	During exposure to low temperature.		During subsequent 3 days at 18° C. on sugar.	
	Coleoptiles.	Roots.	Coleoptiles.	Roots.
6	3.8	2.9	4.7	11.2
8	4.3	1.9	3.9	20.7
12	4.1	2.3	2.3	22.9
16	3.2	2.3	3.0	7.5

Renewed growth of the coleoptiles declines slightly with increased duration of starvation (cf. Table I), but the behaviour of the roots is quite different, and indicates a closer relationship with the progress of vernalisation.

In 1943 the effects of duration of vernalisation in relation to date of sowing were studied, using both whole grain and excised embryos on 2 per cent. sucrose agar. The interaction of the two factors mentioned will be discussed in a later paper, but the experiment included, for comparison, excised embryos vernalised on agar without sugar and the data obtained from these are here presented in Fig. 2 as the weighted mean scores for the three sowing dates used.

With sugar present the results fall on a sigmoid curve as already described in an earlier paper (Purvis, 1944). Without sugar the curve rises slowly throughout, without any definite point of inflection. The further course of the curve is in doubt; in itself it suggests that with prolonged cold treatment the presence of an external sugar-supply is quite immaterial, and that a

maximal effect identical with that obtained with sugar in much less time is possible, but the data from 16 weeks' treatment, given in Table X, suggest rather that the maximal value for the effect of cold without added sugar would fall short of this point. It is, however, quite clear that the presence of sugar in the external medium is not essential for vernalisation, but that it serves to accelerate the process. This does not, however, necessarily mean that sugar

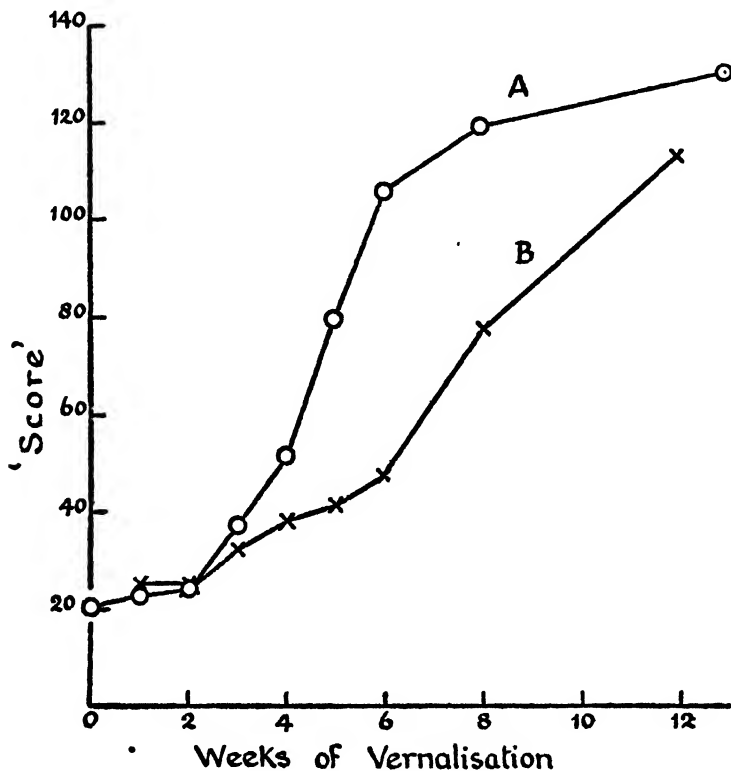


FIG. 2. Excised embryos of Petkus winter rye. The effect of sucrose on the progress of vernalisation. Curve A, 2 per cent. sucrose present throughout given period at low temperature; curve B, low-temperature treatment given without sugar in the medium. Ordinates: mean 'scores' in conventional units. Abscissae: duration of low-temperature treatment in weeks.

plays a subsidiary role in the reactions concerned; possibly it is slowly mobilized in the embryo from polysaccharide reserves other than starch in the cells.

DISCUSSION

1. Effects of 'residual growth' treatment

The interpolation of a period of starvation at room temperature, before the vernalisation treatment of excised embryos, was employed to eliminate residual carbohydrates from the embryos in order that these reserves might not contribute to the vernalisation process. In this way it was expected that more consistent experimental results would be obtained. That such carbo-

hydrates are available in the embryos is demonstrated by the appreciable growth of both coleoptiles and roots in embryos maintained on a medium without sugar at room temperature (Tables I and II). These increase in length from three to four times in 4 days. At low temperature, however (i.e. during vernalisation treatment on 'nil' medium without previous 'residual growth' treatment), much less extension occurs even during more prolonged treatment (Table XI). In this table it may be seen that growth is not only less than that in Tables I and II but that it has ceased after 16 weeks. Moreover, an earlier experiment (Purvis, 1944, Table XIII) showed that on 'nil' medium, no resumption of growth took place after exposure to cold until the embryo had been exposed to light. It cannot be doubted that the stored carbohydrates which promote 'residual growth' at 20° C. are used at 1° C. in the vernalisation process. Thus it is not surprising that when they are removed by starvation the average acceleration resulting from 6 weeks' vernalisation without sugar is substantially reduced (Table VII). Thus the main objective of the treatment was achieved.

It appears, however, that during the starvation period at 20° C. processes other than the elimination of sugar occur. First the ability to utilize sugar for growth is progressively diminished as the 'residual growth' treatment is prolonged (Tables I and II). This depletion of the embryo continues after the visible extension growth has ceased, and results in an embryo which fails to respond at all either to sugar or to sunlight. This final condition demands in some cases more than 4 weeks' starvation.

There is also a retarding effect on flowering. It is not clear from the data available if this retardation is progressive or if it attains a maximal value after about 1 week's treatment (Table VI). The treatment has little effect on the progress made to flowering of winter rye when unvernalsed or vernalised for ineffectively short periods. Indeed Fig. 1 and Table IX show that the 'score' is in some instances slightly increased by the treatment, but this is in no case significant. Spring rye is also retarded by starvation treatment (Table V), but the effect is of much greater magnitude in vernalised winter rye. Thus there appears to be a general retarding effect on the plant, possibly due to a diminished growth-rate, and in the case of winter rye an inhibition also of the process of vernalisation.

These effects of starvation, other than that on vernalisation, are clearly unrelated to the elimination of carbohydrate from the embryo since they are not annulled even when sugar is added at its optimal concentration. Thus during 'residual growth' treatment either some other food substance disappears or some component of the complex of reactions utilizing sucrose is destroyed. This loss may be due to the exudation from the embryos noted, to respiration, or the loss of turgor may play some part. In the absence of direct evidence one may conjecture either a breakdown in nitrogen metabolism or the destruction of a stored hormone, such as auxin. In this connexion the shortening of the coleoptile and of the first leaf, in spring rye as well as in winter rye, is not without significance.

2. *Use of 'residual growth' treatment in vernalisation experiments*

The use of the method in determining the effect of sugar concentration on the vernalisation of excised embryos has in no way altered the conclusion stated in an earlier paper that very low concentrations of sugar suffice for vernalisation. Though the method has been successful in eliminating the sporadic vernalisation of some individual embryos in the absence of sugar, it has not served to establish a clearly defined critical concentration in the medium, though this is shown to be less than 0.5 per cent. sucrose.

A repetition of the transference experiments, described in a previous paper (Purvis, 1944), in which sugar was made available during only part of the vernalisation, but now using the 'residual growth' treatment, has in no way altered the conclusions then drawn, namely, that the vernalisation process is autocatalytic, and that in the absence of endosperm there is a lag period before the process begins. These experiments show, however, that in earlier work the importance of removing residual sugars was overstressed and that even in their absence some vernalisation occurs on a medium free from sugar.

3. *Vernalisation in the absence of free sugar*

An unstarved embryo germinating on a medium containing sugar has available the added sugar in the medium and also that which is rapidly mobilized during germination from its carbohydrate reserves. These together may be termed 'free sugar', and only in embryos subjected to 'residual growth' treatment are free sugars entirely absent. In the previous paper in this series (Purvis, 1944) the experimental results presented indicated that there is a direct effect of cold on the embryo apart from reactions in which free sugar participates. The experiments reported in this paper entirely substantiate this conclusion. Table X and Fig. 2 show that when the low-temperature treatment is prolonged a considerable degree of vernalisation is effected in the absence of sugar in the medium. This prolonged cold cannot be applied after 'residual growth' treatment as the embryos do not survive; there is thus no conclusive direct evidence that this slow vernalisation is not again solely due to residual sugars. But it seems, from the early cessation of growth, that the residual sugars, when present, are used up early in the vernalisation process, and that from the high variance they cause (Table VII) they are not uniformly distributed among different embryos. On the other hand, it can be seen (Table X) that the standard errors of the mean scores become smaller as the treatment is prolonged, the uneven distribution of residual sugars having progressively less effect. It might be inferred from this that this slow vernalisation in the absence of added sugars is not related to the residual carbohydrate, and that vernalisation is not dependent on the presence of free sugar, but progresses slowly in its absence. Unfortunately, for the reason given above, it cannot be stated with certainty what effect complete removal of carbohydrate reserves would have on vernalisation. The curve in Fig. 2 for vernalisation on a medium without sugar is quite clearly autocatalytic and in this respect confirms that there is a direct effect of cold on the embryo apart from the effects of free sugar.

Free sugar should therefore be regarded as an accelerator of vernalisation rather than the determining factor. It does not necessarily follow from this that sugars play no part in the reactions involved. It may well be that in the absence of sugar other storage material in the embryo is slowly mobilized to provide the necessary substrate.

SUMMARY

Residual carbohydrates may be eliminated from excised embryos by a period of starvation at room temperature, a treatment referred to as 'residual growth' treatment.

When this treatment precedes vernalisation on media containing little or no sucrose the variability of the response of the embryos due to their unequal carbohydrate content is reduced. The application of this method to earlier experiments shows that the conclusions drawn from them remain valid.

Though a concentration of sugar in the medium critical for the vernalisation process could not be determined, it is clear that it is not above 0.5 per cent. sucrose. A repetition of the transference experiment described in an earlier paper confirms the suggestion there made that, in addition to reactions in which both low temperature and free sugar are involved, a direct effect of low temperature on the embryo also takes place. This is to some extent substantiated by the continued slow progress of vernalisation in embryos not supplied with sugar in the medium; though in these experiments 'residual growth' proved lethal when followed by prolonged vernalisation without sugar, so that the progress of vernalisation in the entire absence of free sugar still remains in doubt.

During the starvation period not only are the stored carbohydrates eliminated, but the embryos gradually lose their capacity for utilizing sugar for growth and vernalisation.

The author wishes to thank Prof. F. G. Gregory for his stimulating advice in the course of this work. The experiments described here were carried out at East Malling Research Station and the vernalisation treatments at Ditton Laboratory. The author desires to thank Dr. R. G. Hatton and Dr. C. West for the facilities so generously placed at her disposal.

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Studies in the Development of the Inflorescence

II. The Capitula of *Succisa pratensis* Moench. and *Dipsacus fullonum* L.

BY

W. R. PHILIPSON

With Plate I and four Figures in the Text

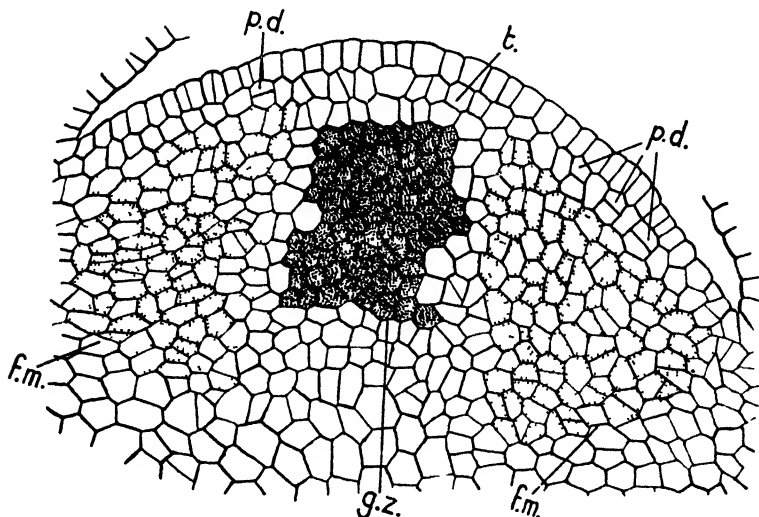
I. *Succisa pratensis* Moench.

ALL the primordia formed at the apex of the main axis of the plant develop into the foliage leaves of the winter rosette. The inflorescences are borne on lateral branches, which perennate as small buds in the axils of the two innermost rosette leaves. The outer leaves of the rosette subtend very minute buds, which, in the normal course of events, remain undeveloped. The leaf-rudiments which form the bud at the centre of the rosette develop during the summer into the rosette of the following winter. Commonly the first leaf-pair of the season subtends well-developed buds which may, in strong plants, develop into a second pair of flowering branches. A single plant, therefore, normally bears one or two pairs of opposite flowering-branches each summer. These lateral branches die back after flowering, so that the plant persists as a monopodial axis, which rots away below as fast as it grows above.

In the dormant state the meristematic apices within the two lateral buds are broad and very flat, a condition commonly found in perennial herbs at a stage when growth is inactive. On the resumption of growth the apex becomes more strongly arched, until it reaches the condition typical of the actively elongating lateral branch. Owing to the simultaneous formation of two opposite leaf-primordia the form of the apex is more symmetrical than that of *Bellis* (Philipson, 1946). The leaf-primordia form farther from the centre of the apical meristem than in *Bellis*, and for a brief period are over-topped by the more strongly arched apex. The apex continues in this condition for some time, forming a succession of primordia which develop into foliage leaves, in whose axils buds form but do not develop.

The distribution of cells at the apex of a lateral shoot in the vegetative phase is shown in Pl. I, Fig. 1, and Text-fig. 1. The tunica is two layers in depth, but the regularity of the periclinal divisions in the second layer is disturbed at the seat of origin of the leaves. The section figured passes through an apex which is about to form a pair of leaf-primordia; the divisions initiating these primordia can be seen in the second tunica layer to either side of the apex, but more particularly to the right, where the folding of the surface to form the primordium is also visible (Text-fig. 1, *p.d.*, and indicated by *X* in Pl. I, Fig. 1). The form of the apex is extremely like that of *Syringa*, figured

and described so fully by Louis (1935), but, even allowing for the decussate, or rather the bijugate (see Church, 1904), phyllotaxis, the zonation of the meristematic cells is dissimilar to that described for the vegetative apex of *Bellis* (Philipson, 1946). The cells on either side of the apex form a distinct flank meristem (Text-fig. 1, *f.m.*, and indicated by arrows in Pl. I, Fig. 1), and it is from this zone that the leaf-primordia are about to develop. The flank meristem surrounds a central zone which consists of a mass of small granular cells (Text-fig. 1, *g.z.*), above which the extreme apex of the axis is



TEXT-FIG. 1. Outline drawing of the cells of the vegetative apex shown in Pl. I, Fig. 1.
f.m., flank meristem, *g.z.*, granular zone; *p.d.*, periclinal divisions, *t*, tunica

formed by a small group of cells which are approximately of the same size as those of the flank meristem. The zonation is, therefore, different from that of *Heracleum* (Majumdar, 1942) and *Bellis*, in both of which species this meristem encloses a central zone of larger cells. Sections through the apices of young lateral buds, which still retain the broad, flat apex found in the dormant bud, show the typical zonation, with flank meristems enclosing a central zone of only slightly larger cells. Another feature distinguishing the active vegetative meristem of *Succisa* from that of *Bellis* is that the filemeristem, or young pith, has not been seen to originate from a cambium-like zone in any of the sections examined.

The flower-heads of *Succisa pratensis* terminate the lateral branches, and, in addition, flowering-branches are borne in the axils of one or more of the leaf-pairs below the terminal head. The inflorescence may, therefore, be described formally as a cyme (bi- or multi-parus) of capitula. The first sign of the formation of this inflorescence is a pause in the production of leaf-primordia while the apex continues its growth, being transformed from a

simple dome into a cylindrical body with a dome-shaped apex. The disposition of the cells in the inflorescence-primordium is shown in Pl. I, Fig. 2. The meristematic tissue has decreased in depth, particularly by cells of the central granular zone passing rapidly into the enlarged state. It is this increase in volume of the central tissue which appears, by distending the apex, to have formed the inflorescence-rudiment. The result is that the whole arch of the apex is enclosed in a uniform peripheral meristem.

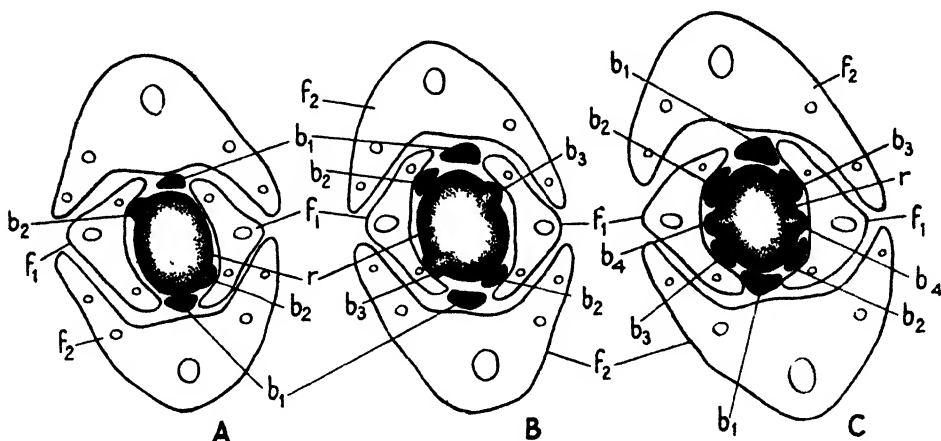
As in *Bellis*, the inflorescence-rudiment becomes divided into peduncle and receptacle by the appearance of the primordia of the involucre bracts. The first bracts begin to form when the rudiment is 150μ high. In *Bellis* the bracts are initiated well below the apex of the rudiment, but in *Succisa* they appear in much the same position as the primordia of the foliage leaves. Pl. I, Fig. 4, represents an inflorescence-rudiment on which bract-primordia are just appearing, the section passing through the second pair of bracts which are themselves scarcely discernible although their provascular strands are clearly defined. Comparison with Pl. I, Fig. 1, shows that the bract-rudiments bear a similar relation to the apex as do primordia of foliage leaves, except that they are raised on a rudimentary peduncle.

It can be seen from Pl. I, Fig. 3, that the second tunica layer undergoes periclinal divisions in the formation of a bract-primordium.

The individual bracts, like the foliage leaves, arise in pairs, and this arrangement is continued through the receptacular scales. Indeed, any distinction between involucre and receptacular scales is a difficult one to draw, because all, even the outermost pair, subtend flower-primordia. The first pair of bracts of the terminal capitulum falls into the position continuing the series of vegetative leaves, but the angle between the diameters on which the first and second pairs of bracts stand is not even approximately that between the successive pairs of foliage leaves, namely 75° . There is a transition from the $2+4$ arrangement of the leaves to the $16+26$ arrangement of the florets, but the details of this transition do not correspond with those figured for *Dipsacus fullonum* by Church (1904, Figs. 64, 65, and 66). By an examination of transverse and longitudinal sections of inflorescence-rudiments in which varying numbers of bracts have been formed it was possible to ascertain the order in which the pairs of involucre bracts are initiated. If the first pair of bracts are considered to be on a diameter running north and south, the second and third pairs lie on diagonals running approximately north-west to south-east (Text-fig. 2A) and north-east to south-west (Text-fig. 2B), and the diameter of the fourth pair crosses that of the first at right angles, running east and west (Text-fig. 2C). These first eight bracts form a symmetrical whorl; they may be regarded as the involucre because they are considerably longer than the bracts which follow, and they enclose the remainder of the receptacle. When the vascular structure of the bracts has been described, however, it will be seen that a few of the succeeding pairs of bracts have some claim to be regarded as part of the involucre. The order of initiation of the subsequent bracts cannot be followed, but proceeds in a general acropetal manner.

At the stage when the involuclral bracts are being laid down the buds in the axils of the uppermost leaves, which eventually develop into lateral capitula, are still rudimentary (Pl. I, Figs. 3, 5, and 6).

The development of the vascular supply of the involucre can be understood most readily after the condition in the mature capitulum has been described, and that description in turn must be based on the vascular supply to the foliage leaves. The vascular bundles in the internodes of a vegetative shoot unite to form a continuous ring of vascular tissue. At each node the six traces



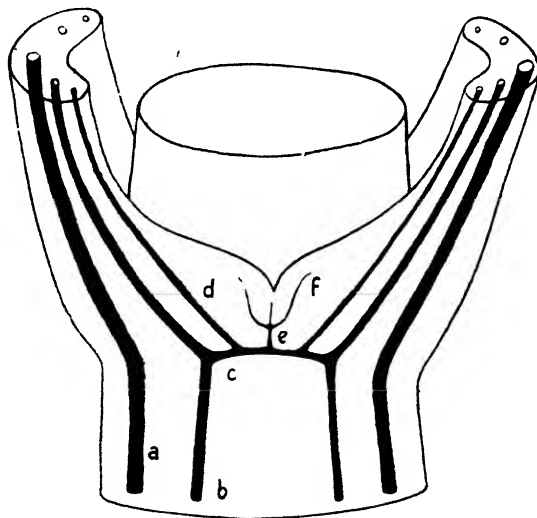
TEXT-FIG. 2 Transverse sections through successively more advanced capitula of *Succisa pratensis*, to illustrate the order in which the bract primordia appear A, with 2 pairs, B, with 3 pairs; C, with 4 pairs, of bract primordia b_1, b_2, b_3, b_4 , successive pairs of bract primordia, numbered in the order of their formation, f_1, f_2 , the two uppermost pairs of leaves, r , receptacle

which supply the pair of leaves separate from the stele, leaving two trilacunar gaps (Sinnott, 1914) which soon become closed by the reconstitution of the vascular ring. Of the three traces to each leaf (Text-fig. 3), the midrib (a) runs through the petiole without branching; each lateral trace (b), however, while still within the petiolar sheath, is joined to its fellow from the other leaf of the pair by a more or less horizontal branch (c). This horizontal vein gives off three vertical veins; one enters each petiole (d), the third (e) is common to the two leaves. Just below the cleft where the sheath divides into the two leaves this common vein gives off to right and left a marginal vein (f) to each leaf, but continues upwards for a short distance as a minute vein common to the two leaves.

The vascular ring is reconstituted above the uppermost pair of vegetative leaves, and continues unchanged throughout the peduncle. At the level of the involucre eight traces leave the stele, forming gaps. They are the midribs of the eight bracts of the involucre. Each gives off strong lateral branches to right and left, which curve to meet their fellows from the adjacent bracts (see Text-fig. 4). This arrangement might be derived from that of the foliage leaves if the laterals and midribs of each foliar organ were considered to have

united, but it must be borne in mind that the vascular connexions in the involucre are not between members of the same whorl.

Each of the arched transverse veins gives off from its distal edge a series of veins which run up into the blades of the two bracts which it connects. The number of these lateral veins in each half of an involucral bract is normally three or four. It can be seen from the figure that the eight mid-ribs are intimately connected by the transverse veins to form a closely knit vascular system supplying the involucre. Lateral connexions between bracts are not

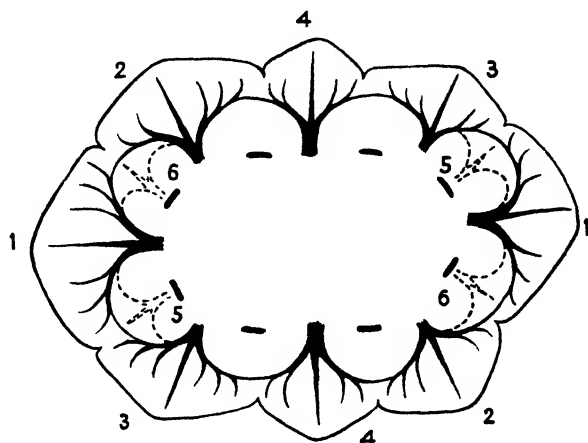


TEXT-FIG. 3. Diagram of the course of the vascular bundles at a node of *Succisa pratensis*.
For description see text.

confined to the involucre, however, for a varying, but very small, number of subsequent pairs of bracts are linked to the involucre by the union of their lateral veins. Such connexions are usually confined to the two pairs of bracts succeeding the involucre, and their connexions are made with the lateral veins of the outermost pair of bracts (see Text-fig. 4). The later-formed bracts have a similar vascular system, with a median trace, and two lateral branches. The latter, however, run into the blade of the bract without meeting the vascular system of any other bract. The vascular system of all bracts is, therefore, fundamentally the same, but those of the involucral bracts are connected through their lateral branches.

The course of development of the vascular system of the peduncle and involucre is similar to that described for *Bellis*. A cylindrical provascular meristem is formed at the base of the inflorescence-rudiment by the maturation of the middle layers of the peripheral meristem. The peripheral meristem itself is an extension of the apical meristem down the flanks of the inflorescence-rudiment: the provascular meristem is, therefore, continuous with the apical meristem. The cleft in the peripheral meristem which forms

the provascular meristem is shown in Pl. I, Fig. 3. The provascular cylinder develops into the stele of the peduncle, becoming more strongly developed in the position of the midribs of the eight involuclral bracts. The maturation of the middle layer of the peripheral meristem is checked in the position of each bract-primordium, so that the vascular meristem within the bract retains connexion with the central stele. In Pl. I, Fig. 3, the bract-rudiment and the provascular meristem can be seen to be united by continuous meristematic tissue.



TEXT-FIG. 4. Diagram of the vascular system of the involucre of *Succisa pratensis*. The four pairs of involuclral bracts are numbered in the order of their initiation. The lateral traces of adjacent bracts anastomose. The vascular system of receptacular scales which are similarly connected to the involucre are shown in broken outline.

The involuclral bracts are provided with no vascular strands other than their midribs until they are about 300μ in height. By this time the receptacular scales are beginning to form, and flower-primordia are appearing in the axils of the outermost involuclral bracts (Pl. I, Fig. 6). The two lateral veins of each involuclral bract appear as branches of the midrib; they do not form from provascular meristem but by the secondary meristemization of tissue which has already undergone maturation. By the time the elongated receptacle is covered with bracts and flower-primordia, but before primordia of floral organs have begun to appear on even the most advanced primordia (Pl. I, Fig. 7), the lateral veins of adjacent bracts have united and the principal strands which serve the lamina of the bract have developed from them (see Text-fig. 4).

With the appearance of the last involuclral-bract primordia the buds in the axils of the uppermost foliage leaves, which previously have slowly increased in size, develop the primordia of their prophylls. The primordia of the involuclral bracts immediately succeed the prophylls, and the development of these lateral capitula proceeds on the same lines as that of the terminal capitula.

The receptacle grows in breadth as well as in height by the action of the hemispherical peripheral meristem. The mass of the central pith is increased not only by the addition of cells from the apex and the growth and division of cells within itself, but also by the addition of cells laterally. The continual conversion of the inner cells of the peripheral meristem to pith cells prevents any great increase in the depth of the meristem.

The swellings on the surface of the receptacle which become bract-primordia have their origin in divisions of the second and third cell-layers. These bract rudiments grow rapidly in length and can be dismissed with no more comment than that they cover the abaxial surface of the flower-primordia during their development, and that each receives a single vascular strand which later gives off branches to right and left in its lamina. In the axil of each bract the peripheral meristem persists as an axillary bud which soon develops a hemispherical shape. The origin of the bract and the flower-primordium, with their vascular supply, can be seen more clearly in *Dipsacus fullonum* and will be described more fully for that species in the second part of this paper.

The primordium in the axil of each receptacular scale gives rise not only to the floral organs but also to the structure known as the outer calyx. This structure can be seen as a fold of tissue completely encircling the floral rudiment. It is inserted obliquely on the pedicel, being higher abaxially than adaxially (Pl. I, Fig. 8). At four zones around this outer calyx the meristematic tissue is particularly dense; these zones are situated abaxially and adaxially in the median plane and symmetrically to right and left. These zones evidently represent the primordia of four members of the outer calyx; as in the whorls of the floral organs the primordia arise simultaneously. The development, therefore, gives no clue as to which of the members of the outer calyx (if any) are to be regarded as prophylls (see Doll, 1927).

The floral rudiment above the insertion of the outer calyx gives rise to the normal floral organs and envelopes.

II. *Dipsacus fullonum* L.¹

The development of the capitulum of *D. fullonum* does not differ essentially from that of *Succisa pratensis* and it is not proposed, therefore, to describe it fully. This type is particularly suitable, however, for showing the early stages in the initiation of floral bracts and flower-primordia, and it is to these features that the following description will be confined.

In *D. fullonum*, unlike *S. pratensis*, the main axis of the plant bears capitula, and, as no lateral buds persist, the plant is monocarpic. The plant is biennial, perennating as a rosette of leaves around a central axis, which, in spring, elongates into a tall, branched flowering-stem. At the end of the winter period of dormancy the vegetative axis has a very broad apex. The tunica is two layers deep and encloses a corpus comprising a central zone surrounded by a smaller-celled flank meristem. Following the rapid elongation of the

¹ The true *Dipsacus fullonum* of Linnaeus is the *D. sylvestris* of Hudson.

stem in spring, the vegetative apex becomes more strongly domed, until the production of leaf-primordia ceases and the apex is transformed into the primordium of the capitulum.

The formation of the involucre and its vascular supply, the development of lateral flowering branches, and the growth in breadth of the receptacle are all essentially as in *Succisa*. The initiation of the floral bracts and the flower-primordia are also essentially as in *Succisa*, but in view of the clarity with which the early stages can be seen in this material, they are described fully in order to supplement the description of *Succisa*.

The inflorescence-rudiment is oblong in longitudinal section, the peripheral meristem extending evenly across the flat top of the rudiment and some way down the flanks. The outer two layers of this meristem form a regular tunica, within which further meristematic cells are irregularly arranged. The primordia of the bracts and flowers appear on the almost vertical flanks of the inflorescence rudiment. The first indication of their position is an increase in the size and density of staining of the tunica cells and of the cells immediately below it (Pl. I, Fig. 10, indicated by arrow). Anticlinal, as well as periclinal, divisions in the second tunica layer and the cells below lead to the development of a swelling, which is covered by a single tunica layer (Pl. I, Fig. 11, at (b)). This swelling is the primordium of a bract; its cells remain meristematic but the peripheral cells of the inflorescence-rudiment both above and below it lose their meristematic character, except for a small group in the axil of the bract, where a residue of the peripheral meristem remains (Pl. I, Fig. 11, at (f)). This group of cells is the first indication of the primordium of the flower. Cell-divisions are frequent in this group, both in the two tunica layers and in the cells below, but at first all divisions are anticlinal, forming a plate of narrow cells. The divisions in the two tunica layers remain anticlinal, so that even at an advanced stage the flower-rudiment is enclosed in a two-layered tunica. The flower-primordium comes to project beyond the surface by reason of divisions in its inner cells, which now take place in all directions. The meristematic cells of the flower-primordium are no longer in contact with those of the bract-primordium, because the adaxial cells of the latter have become vacuolated (Pl. I, Fig. 12); the primordium of the flower is in fact an isolated meristem surrounded, except to the outside, by maturing tissue.

The provascular meristem of the bract develops from the peripheral meristem as in *Succisa* and in the floral-primordia of *Bellis*. The section figured in Pl. I, Fig. 10, passes through the peripheral meristem at a point where the primordium of a bract is being initiated. Above the level of the incipient primordium the peripheral meristem is homogeneous; below it the central layers of the meristem have become vacuolated so that a provascular meristem is divided from the remainder of the peripheral meristem. It can be seen clearly that the provascular meristem is in contact with the apical meristem of the inflorescence-rudiment; it retains this contact by acropetal development as further primordia are defined, its upward growth

following the spiral of the short parastichy on which the bracts lie. Similar, but more slender, provascular strands follow the long parastichies. A mid-rib is formed in the bract as it increases in size (Pl. I, Figs. 11 and 12) and the provascular meristem of this bract-trace is in continuity, from its inception, with that in the stele of the inflorescence-rudiment. That is, the provascular network of the inflorescence-rudiment and the bract-traces develop in a continuous acropetal manner and are formed from a meristem which is directly descended from the apical meristem.

The origin of the strands which supply the flowers is rather different from that just described. It has already been stated that the flower-primordia originate in the axils of the bracts as residues of peripheral meristem, which begin to divide actively immediately after the inception of the bracts. These axillary meristems are particularly clearly defined, and an examination of a series of sections demonstrates that, at the stage shown in Pl. I, Fig. 12, they become separated from the bract trace and from the network of strands in the inflorescence-rudiment, with both of which they were originally in contact (see Pl. I, Fig. 11). As the lobes of the outer-calyx, and later of the floral organs, are initiated, provascular meristem is defined from the meristem of the primordium of the flower, and it is evident that this provascular tissue can come into contact with the vascular system of the main axis only by the development of strands bridging the previously vacuolated tissue. The flower-traces, therefore, are not derived directly from the apical meristem, but arise by the secondary meristemization of partially matured tissue. The provascular tissue of the floral axis forms a cylindrical, hollow stele (see Pl. I, Fig. 8, in the case of *Succisa*). Before this stele is inserted on that of the inflorescence axis it is resolved into a pair of bundles placed transversely above the trace of the bract. These two 'bud-traces' diverge as they pass inwards, one being inserted on each side of the 'leaf-gap' of the subtending bract. This form of insertion appears to be very general in axillary branches, whether leafy or floral. Pl. I, Fig. 13, represents the rudiment of a flower cut obliquely so that the section passes through the median part of the floral apex (e.g. the two median lobes of the outer calyx are cut), but in the inflorescence axis it has been deflected to one side so that the insertion of one of the flower-traces on the bundle which flanks the 'leaf-gap' is shown (cf. also Pl. I, Fig. 9).

DISCUSSION

The type of zonation visible within the apical meristem of the lateral branches of *Succisa* when undergoing active leaf-production was found to differ from that shown by other dicotyledonous types investigated. The zonation which *Succisa* apices show earlier in the season, that is immediately they become active after remaining dormant through the winter, is more typical, with the flank zone enclosing a large-celled central zone. In *Bellis* (Philipson, 1946) it was shown that the reproductive form of the apex was derived from the vegetative form by a readjustment of the meristematic zones, and it is suggested that in *Succisa* the form of the apex changes progressively

from that shown in the dormant bud to that characteristic of the young capitulum, and that the leaf-primordia are laid down on an apex intermediate between the two extremes. As all the buds which develop on the lateral branches of *Succisa* form capitula the whole branch might be regarded as an inflorescence and the intermediate form of the apex be thus explained.

In describing the development of the flower of *Delphinium* and of its subtending bract, Grégoire (1938) considers that they do not bear the same relationship to one another as do a leaf and its axillary branch. He considers that the two structures, bract and floral apex, originate as a single primordium, the flower developing from the upper face of the bract, and he supports this view by stating that the mature flower and bract appear to be inserted on the inflorescence axis as a unit. Grégoire cites Warming (1872) who published many figures showing the close relationship between leaves and the buds in their axils, but he did not draw any distinction between leaves and bracts in this connexion, for he regarded the vegetative leaf and its bud as much a unit as the bract and its flower. Grégoire further considers that the zonation of the meristem of the floral apex is that typical of reproductive apices, which, in his view, is fundamentally distinct from that of vegetative apices. The development of the bracts and flowers of *Dipsacus*, as described and figured in the present paper, show, in the opinion of the author, that the floral apex has a distinct origin from that of its bract, and that the form and development of the vascular strands supplying the bract and the flower suggest that these structures are related in the same way as leaf and axillary bud. The work of Reeve (1943) on *Garrya* also brings out the essential similarity of vegetative buds and axillary flowers.

The origin of the vascular supply of the flower is interesting. The floral primordia are formed by cells of the peripheral meristem retaining their meristematic character while the surrounding tissue becomes vacuolated. As the primordia grow provascular tissue is formed within them, but their vascular system can only become united with that of the main axis by the development of provascular strands from previously vacuolated tissue. Reeve (1943) describes the development of the provascular strands of axillary buds as continuously acropetal, but does not state whether the origin of the strands is from a primary or secondary meristem. Wardlaw (1943) describes the development of isolated residues of the apical meristem in the fern *Dryopteris felix-mas*, in which the vascular systems of the lateral axes remain unconnected with the central stele. A somewhat parallel development is described for the pinnae of the fronds of *Zamia* (Johnson, 1943), where the primordia of the leaflets appear in advance of their provascular strands, which eventually develop outwards towards them from the midrib of the frond. In the present work it has not been possible to ascertain whether the provascular strand of the floral axis develops acropetally from the central stele, because the bridge between the primordium and the stele forms quickly and apparently simultaneously across the whole width of the gap. The subsequent development of the floral trace is acropetal.

The outer calyx is very characteristic of the family and has been closely studied. Doll (1927) reviewed the various interpretations of this structure. He himself considers it to be formed by the fusion of two bimerous whorls of bracts, of which the lower whorl is placed medianly. He states that in *Morina* the median primordia appear earlier than those of the transverse pair, though this may be due to their being considerably the larger when mature. He considers that true prophylls are absent. The present study throws no light on the presence or absence of prophylls in the outer calyx, but, anatomically, its two transversely placed members act as prophylls, for it is by their traces that the vascular system of the floral axis is inserted on either side of the leaf-gap of the floral bract.

A comparison of the development of the capitulum in the Compositae and Dipsacaceae will not be made until further types have been described. However, some comparison of *Bellis* with *Succisa* may be of interest. There can be no doubt that the course of development is fundamentally the same in both types, that is, they show a similar transition from the zonation of the vegetative apex to that of the reproductive apex, the method of increase in size of the receptacle is the same, and the provascular tissue originates in the same way. But it is probable that these features are fundamental to the development of structures of this type, and that any comparison with a view to assessing relationship should concern itself with details rather than fundamentals.

In the first place it may be useful to examine more closely the concept of a capitulum. If no more is meant than an aggregation of sessile flowers on a short receptacle any relationship based on the possession of this character alone is not likely to be well-founded. Schleiden (1849) has pointed out that most inflorescences pass through a capitulate stage in their development, and regards the capitulum as the basic form of inflorescence. As the relationship which some systematists have suggested between the Compositae and the Dipsacaceae rests almost entirely on the occurrence of capitula in the two families, it cannot be regarded as established unless there is more than a general resemblance between the inflorescences, or unless other common characters are found. As regards their inflorescences, firstly, the capitula of the Compositae are characterized by the possession of an involucre of bracts in whose axils flowers do not occur, whereas the involucre bracts of the Dipsacaceae subtend flowers; secondly, the florets of the Dipsacaceae are invested by an outer calyx, a structure unknown in the Compositae. On *a priori* grounds, therefore, there is no reason to regard the capitula as identical structures in the two families, and that being so the principal link between them is broken. A confirmatory character of the inflorescence, further separating the two families, is provided by the floral trace: in *Bellis*, and so far as is known in all Compositae, this consists of a single strand; in *Succisa* and *Dipsacus* it is of the nature of a medullated stele. In a later paper it is proposed to examine in detail the characters, other than those of the inflorescence, which have been used either to unite or to separate these two groups.

SUMMARY

The zonation of the cells of the apical meristem of the lateral branches of *Succisa* is at first similar to that typical of many dicotyledons, comprising a tunica enclosing a corpus with a central zone of large, lightly staining cells, surrounded by a peripheral zone. During the formation of the leaves which precede the formation of the capitula this zonation is altered by the transformation of the central zone into a zone of small granular cells. In the inflorescence rudiment this zone disappears, the peripheral zone then forming a continuous meristem over the dome-shaped apex.

The development of the capitulum of *Succisa* and *Dipsacus* closely resembles that of *Bellis*, particularly as regards growth in volume of the receptacle and the differentiation of provascular meristems. It is suggested that these resemblances are fundamental to structures of this nature, and do not necessarily imply close relationship. It is pointed out that the capitula of the Compositae and the Dipsacaceae are not strictly comparable, (a) because the involucre of the Compositae, in which flower-buds are lacking, has no counterpart in the Dipsacaceae, and (b) because the 'outer-calyx' of the Dipsacaceae is absent from the Compositae.

The course of development of the flowers of *Dipsacus* suggests that the relationship between the floral bracts and the flower primordia in their axils is essentially the same as that between a foliage leaf and its axillary bud.

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DESCRIPTION OF PLATE I

Illustrating Mr. W. R. Philipson's article 'Studies in the Development of the Inflorescence. II. The Capitula of *Succisa pratensis* Moench. and *Dipsacus fullonum* L.'

Figs. 1-9. *Succisa pratensis*

Fig. 1. Longitudinal section through apex of lateral branch during initiation of leaf-primordia. Flank meristem indicated by arrows, with the central 'granular' zone between. Periclinal divisions can be seen in the second tunica layer to right and left of the apex, and at X the folding of the surface to form a leaf-primordium is visible (compare with Text-fig. 1). ($\times 200$.)

Fig. 2. Longitudinal section through apex of lateral branch which has been transformed into an inflorescence rudiment. The apex is covered with a uniform peripheral meristem. Bract-primordia, cut non-medianly, can be seen to right and left. ($\times 200$.)

Fig. 3. Longitudinal section through inflorescence rudiment, with bract-primordium cut medianly. The bract trace, indicated by arrows, has been differentiated from the peripheral meristem by the vacuolation of cortical cells. Note (i) the periclinal division in the second tunica layer at the apex of the bract-primordium, and (ii) the rudimentary bud in the axil of the uppermost foliage-leaf. ($\times 200$.)

Fig. 4. Longitudinal section through inflorescence rudiment. The two provascular strands (at X, X) are continuous with the peripheral meristem, although the bract-primordia to which they correspond are scarcely discernible. ($\times 200$.)

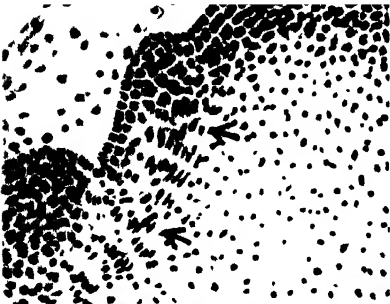
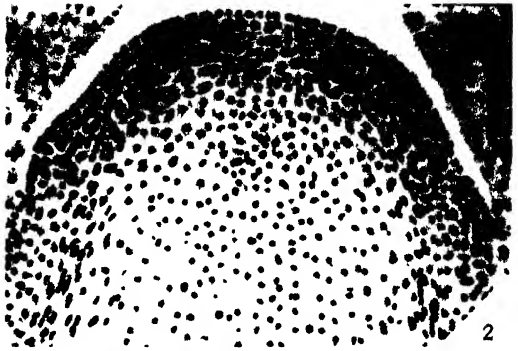
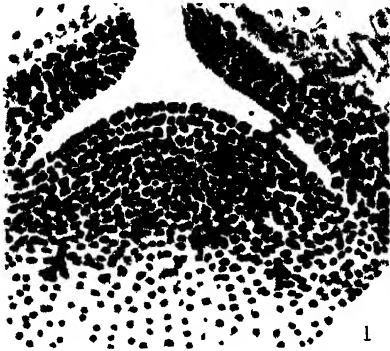
Figs. 5-8. Longitudinal sections through successively older inflorescence rudiments. Fig. 5. Involucre defined, receptacle hemispherical. Fig. 6. Involucre enlarged, initials of floral bracts appearing. Fig. 7. Receptacle covered with primordia of floral bracts and florets. Fig. 8. Rudiments of florets with primordia of floral organs. At X the floral trace can be seen to be a medulated stele, and the median members of the outer calyx are indicated by arrows. ($\times 50$.)

Fig. 9. Longitudinal section of advanced flower-bud. The section is cut obliquely, so that the insertion of one floral trace on to one side of the 'leaf-gap' is seen. This trace gives off strands to the outer calyx (o), calyx (c), corolla (p), and stamens (s), all of which lie in the median plane. ($\times 80$.)

Figs. 10-13. *Dipsacus fullonum*

Figs. 10-12. Longitudinal sections through successively older primordia of floral-bracts and flowers. Fig. 10. The initiation of a bract-primordium is indicated by the arrow. Above this the peripheral meristem is uniform, below it has been differentiated into a provascular meristem and a peripheral meristem by vacuolation of cortical cells. Fig. 11. A single tunica layer encloses a highly meristematic bract-primordium (b), to which a provascular strand is connected. The peripheral meristem above the bract has now become vacuolated, except for some meristematic cells (f) in the axil of the bract. Fig. 12. The bract-primordium (b) shows tissue differentiation, and the cells (f) in its axil are forming a flower-primordium. This well-defined meristematic mass is surrounded by vacuolated tissue. ($\times 500$.)

Fig. 13. Longitudinal section of flower-rudiment. The two median members of the outer calyx have been cut. The section is slightly oblique so that the insertion of one of the floral-traces to one side of the 'leaf-gap' is included in the section. ($\times 110$.)

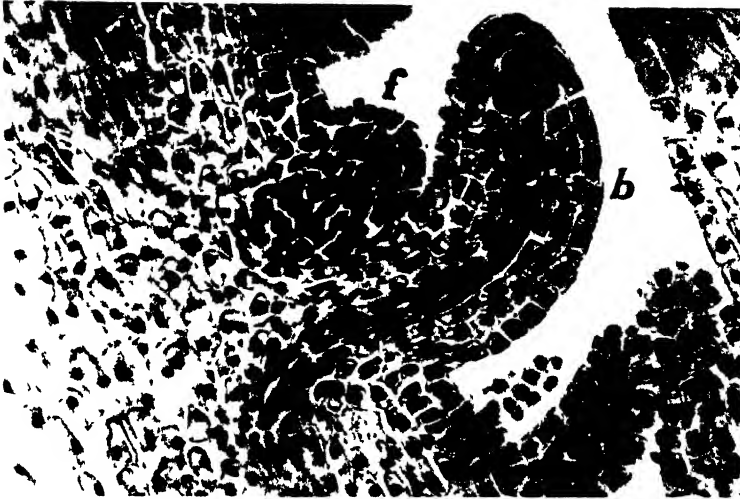




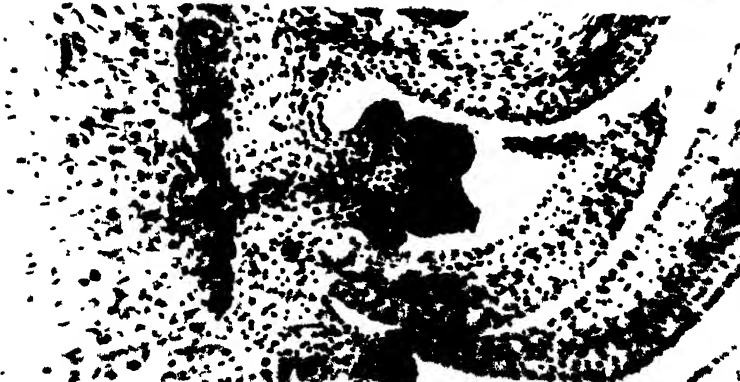
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Studies in Tropical Fruits

XVII. The Respiration of Bananas in Different Concentrations of Oxygen at 53° F., and during subsequent Ripening in Air at 68° F.

BY

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With fifteen Figures in the Text

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I. INTRODUCTION

IN earlier papers of this series the respiration of bananas in air at tropical temperatures (Wardlaw and Leonard, 1940) and at 53° F. with subsequent ripening at 65° or 68° F. (Leonard and Wardlaw, 1941) have been described. The present contribution deals with respiration at 53° F. in different concentrations of oxygen below and above that in air (20.9 per cent.), with subsequent ripening at 68° F. in air. It thus constitutes essentially an approach to the application of storage in controlled atmospheres ('gas-storage') along physiological lines. No attempt, therefore, has been made to produce conditions of anaerobic respiration, and no particular attention has been paid to the effects of transition from air to atmospheres of different oxygen content and vice versa. (Transfer to different oxygen concentrations was made simultaneously with the changes from one to another temperature.) In each experiment the behaviour of the four control fruits, in air at two humidities, provides data on some aspects of the phenomena of 'chilling'. These must await adequate treatment later.

In commercial practice the temperature of the environment of bananas is controlled during both the (overseas) storage and ripening (ashore) phases. Accordingly, in the series of experiments detailed here the customary temperatures have been adopted and the course of ripening followed until a late stage

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in senescence, so that any deviation from what may be described as 'normal' ripening might be observed, and so that the value of the altered storage environment might be assessed in terms of the prolongation of the period prior to ripening.

A considerable number of experiments have been made by investigators in various countries on the storage of bananas in different gas mixtures: in almost all instances, however, these have been made on fruit which had previously undergone an overseas voyage and not immediately after harvesting in the country of production. The majority involved combinations of high carbon dioxide and low oxygen concentrations, but some consisted in varying the oxygen concentration alone (Gerber, 1903; Geerlings, 1908; Kidd and West, 1932, 1933; Gane, 1934, 1935, and 1936; Lynch, 1935; Scurti and Zavanaju, 1936; Zavanaju, 1936; Wardlaw, 1940; and Thornton, 1943). Their results are discussed in relation to the data obtained here.

II. MATERIALS AND METHODS

Single fingers¹ of Gros Michel bananas have been used throughout the experiments, from bunches selected as suitable for overseas shipment, not showing the pulp colour associated with *Cercospora* leaf-spot infection of the plant (Campbell, 1925; Surridge, 1932; Wardlaw, Leonard, and Barnell, 1939) and of grades heavier than '¾-full';¹ the actual grade as assessed by weight and pulp/skin weight ratio is given in each instance. Observations were started as far as possible within 24 hours of harvesting. Owing to war-time difficulties in transport and inexperience of estate labour, the bananas were, apparently, occasionally 'stale' (i.e. fully 24 hours elapsed between harvesting and commencement of observations) and the grade was somewhat variable. The second, third, and fourth hands¹ of a 'count'¹ bunch bear six to ten fingers in the upper row,¹ and, as discussed previously (Wardlaw, Leonard, and Barnell, 1939*a*; Leonard, 1941), these constitute experimental material of considerable uniformity. In these experiments, therefore, eight fingers have been taken from the upper row of a second to fourth hand, the respiration rate of two being determined individually in a chamber supplied with air, and that of two pairs of replicates in chambers supplied with two different gas mixtures, the remaining two being left in the room atmosphere for determination of their internal gas concentrations. This involves considerable repetition of records of the respiratory behaviour of bananas in air, which serve as controls in each experiment. This is, however, unavoidable since (i) the difference in time of ripening of individual bunches, even when selected as uniform material, is very considerable; (ii) the acropetal succession of ripening of the different hands in a bunch (Leonard, 1941) precludes the use of fruit from more than one hand; and (iii) the labour in collecting the necessary data is considerable and records must necessarily be kept up to date if the rapid trends are to be followed. In all instances an atmosphere saturated with water vapour was maintained in the respiration chambers: the relative

¹ For explanation of terms see Wardlaw, Leonard, and Barnell, 1939*a*.

humidity in the room at 53° F. was 80–5 per cent.; but in the last experiment this was increased to 88 per cent. by alteration in the temperature-control mechanism.

The methods used in the estimation of respiration in air were those described in an earlier paper (Wardlaw and Leonard, 1939). The modifications in the apparatus for bananas in different oxygen concentrations consisted in the supply of gas from cylinders containing different mixtures of oxygen in nitrogen through a regulating valve instead of from the outside air. Suction was applied at the outlet end. A manometer was attached at the inlet to the respiration chambers so that the reduction of pressure in the chamber could be adjusted, by controlling the inlet pressure, to approximately that in the chambers supplied with air by suction only; it amounted to 10–15 cm. liquid paraffin.

A period of 19 days from receipt at the laboratory was arbitrarily taken as that for storage at 53° F. This is considerably in excess of the 12 to 14 days of normal commercial practice for this grade of fruit ('Canadian' grade, i.e. more than $\frac{3}{4}$ -full'). It represented the useful life of two 150- or three 100-cu. ft. cylinders of gas used in succession, at a rate of flow of 200 c.c./minute to two tubes. This rate of draw-through had previously been found adequate to ensure a negligible accumulation of carbon dioxide in the respiration chamber and, by inference, of those volatile substances known to stimulate ripening. A maximum of 0.23 per cent. carbon dioxide was recorded on two occasions.

III. RESPIRATION IN OXYGEN CONCENTRATIONS LESS THAN IN AIR

(a) *Respiration in 14 per cent. and 5 to 7 per cent. oxygen.*

The weights of the experimental bunch, hand, and fingers are given below.

Weight of intact bunch (10 hands)	28.45 kg.
Weight of third hand	3.30 "
Mean initial weight of upper row fingers of third hand	179.54 gm.
Initial pulp/skin ratio of one outer finger	1.60 "

On the basis of weight of finger and pulp/skin ratio this bunch was therefore 'heavy $\frac{3}{4}$ -full' (cf. Wardlaw, Leonard, and Barnell, 1939a, Figs. 1 and 2).

Fig. 1 gives the internal gas concentrations for one of the two fingers exposed to the room atmosphere, and the record of temperature and humidity (53° F. and 70 to 80 per cent. R.H., followed by 68° F. and approximately 85 per cent. R.H.). Annotations indicate the various ripening changes observed (Wardlaw and Leonard, 1940).

Comparison may be made with Figs. 12, 13, and 16 (Leonard and Wardlaw, 1941) for similar banana fingers under similar conditions. It will be seen that the fruit remained green and its internal carbon dioxide concentration low and oxygen high until the 12th to 13th day at 53° F., after which a slow rise in carbon dioxide and fall in oxygen concentrations occurred with yellowing of the skin, so that, on transfer to 68° F. on the 19th day, the fruit had progressed a considerable way to its climacteric and a trend of internal gas

concentrations occurred, intermediate between that for fruit maintained throughout at 53° F. (Fig. 16, Leonard and Wardlaw, 1941) and that transferred to 68° F. after 14 days (Figs. 12 and 13, Leonard and Wardlaw, 1941). This abnormality took the form of a lower maximum of carbon dioxide

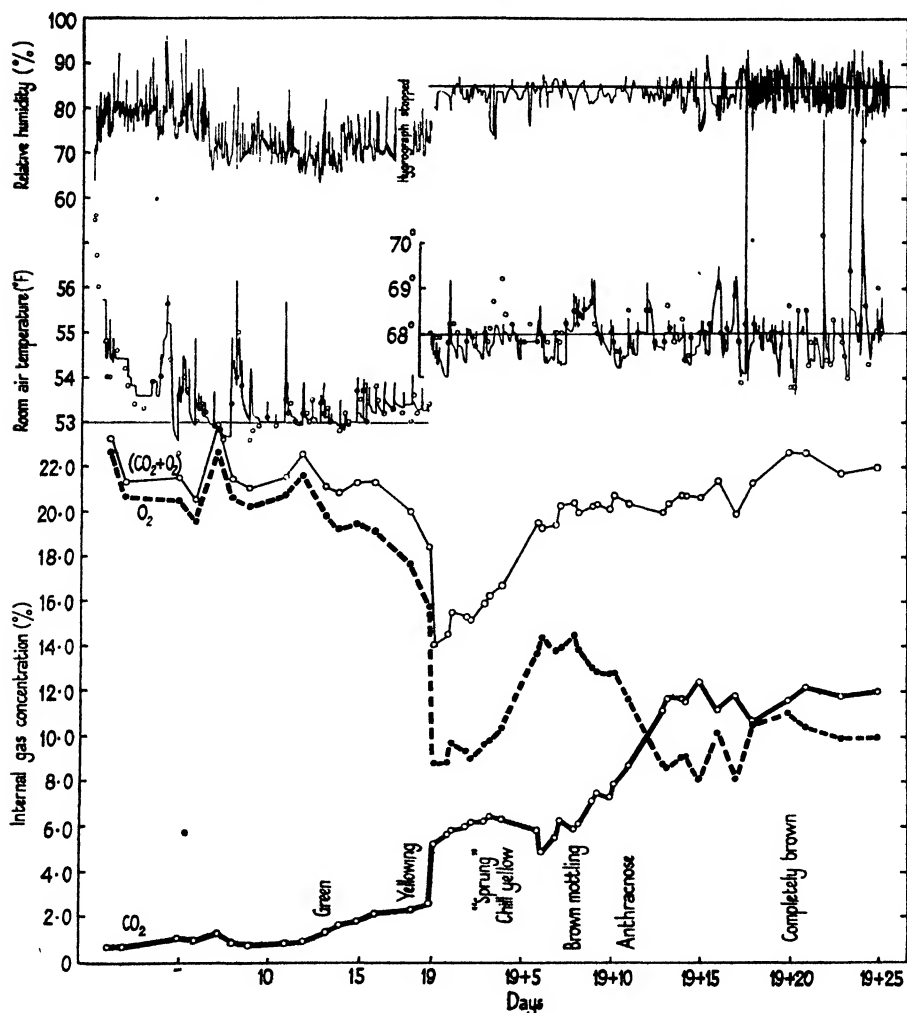


FIG. 1. Internal concentrations of CO₂, O₂, and CO₂+O₂ of a 'heavy ¾-full' banana in air for 19 days at 53° F. and 70-80 per cent. R.H., followed by 68° F. and 85 per cent. R.H. Thermograph and point records of temperature and hygograph record shown.

concentration and a higher minimum of oxygen, a new, additional criterion of the phenomena of chilling (Wardlaw and McGuire, 1931, p. 10). The time of incidence of brown mottling of the skin and of the first appearance of anthracnose was also advanced, and also the final senescent rise in internal carbon dioxide concentration and decrease in internal oxygen concentration. These last, however, did not reach the extreme limits (high CO₂ and low O₂)

TABLE I

Weights, Respiration Data, and External Changes observed in Banana Fingers in Air at 53° F., followed by 68° F.

	Weight (gm.)		Loss (%).	Total days.	Pulp/ skin.	Mean pre-		Days to reach stages given below			
						climacteric int. conc.		Initiation of CO ₂ rise and O ₂ fall.	Full yellow skin.*	Brown mottling.	Anthraxnose.*
	Initial.	Final.				CO ₂ %	O ₂ %				
Finger of Fig. 1	185.84	132.11	28.92	19+25	3.13	1.0	20.6	12	19+5	19+8	19+11
Replicate .	178.83	151.15	15.48	19+17	2.79	1.0	19.0	12	19+5	19+8	19+11

* The period between Full yellow skin and Anthracnose may be taken as marking the limits of the 'eating-ripe' period.

TABLE II

Weights, Respiration Data, and External Changes observed in Banana Fingers in Air at 53° F. followed by 68° F.

	Weight (gm.).		Loss (%).	Total days.	Pulp/ skin.	Mean pre-climacteric		Initiation of climacteric rise on basis of				Days to			Senescent rise in respira- tion rate.
						Int. conc.	Respira- tion rate. (mg./kg./hr.)	(1) Internal CO ₂ rise and O ₂ fall.	(2) Respira- tion rate rise.	Full yellow skin.	Brown mott- ling.	An- thrax- nose.			
	CO ₂ %	O ₂ %													
	Initial.	Final.													
Finger of Fig. 2	191.10	173.95	8.98	19+32	2.27	1.2	17.2	8	12	19+5	19+8	19+11	19+20		
Replicate .	167.35	152.75	8.72	19+28	1.90	1.0	17.8	13	15	19+4	19+8	19+11	19+20		

normally observed in fruit ripening without chilling. Additional data are shown in Table I.

The trend of respiration rate of a finger in air (20.9 per cent. O_2) is given in Fig. 2 together with its internal gas concentrations. It will be seen that a very similar trend of internal gas concentrations occurred in this fruit, although the external environment was different in respect of humidity

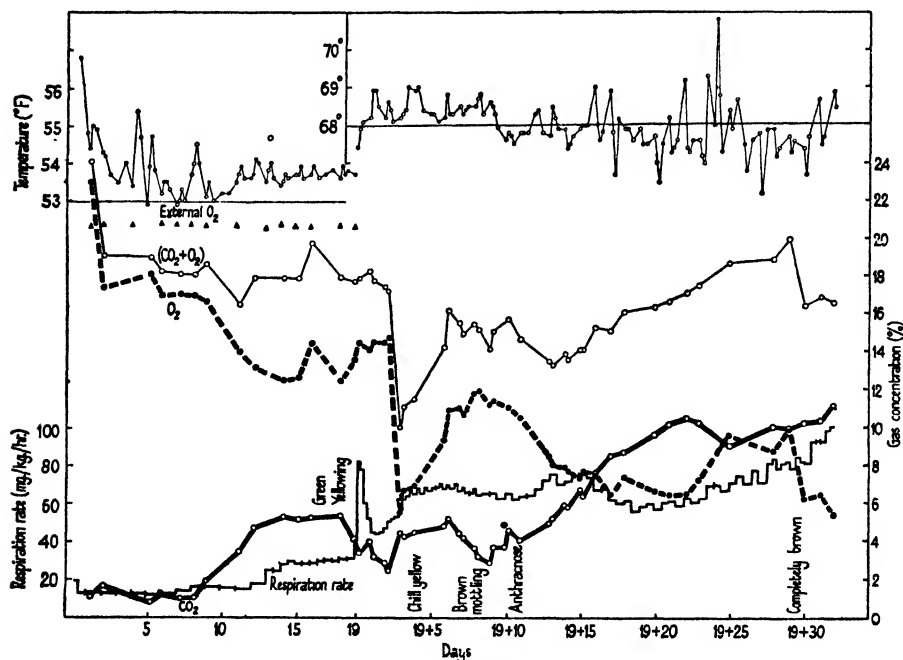


FIG. 2. Rate of respiration and internal concentrations of CO_2 , O_2 and CO_2+O_2 of a 'heavy $\frac{3}{4}$ -full' banana in air (20.9 per cent. O_2) at 100 per cent. R.H. for 19 days at $53^\circ F.$, followed by $68^\circ F.$ External O_2 concentrations at $53^\circ F.$, black triangles. Air-temperature record shown.

(Fig. 1, 70–80 per cent. at $53^\circ F.$ and 85 per cent. at $68^\circ F.$; Fig. 2, approximately saturation throughout). The incidence of the climacteric, however, was somewhat earlier and the transfer to $68^\circ F.$ was made apparently about the climacteric peak. Accordingly, the respiration rate gave a record intermediate between that for fruit continuously at $53^\circ F.$ and that transferred to the higher temperature without chilling having been incurred. A rapid transitory rise in respiration rate occurred on transfer to $68^\circ F.$ Point observations of external oxygen concentration (black triangles) and of the air temperature in the respiration chamber are also given. Table II gives data on the fruit and its replicate.

Except that lower percentages of internal oxygen occurred in the fruit at the higher humidity, the behaviour of these four fingers in air is thus very similar, as regards values and trends of internal gas concentrations prior to the onset of ripening, and as to the time of incidence of the various changes

in the skin coloration and of disease. Whilst slight chill coloration of the skin was evident in all four fingers in the early stages of ripening, the final yellow colour was not markedly abnormal.

Corresponding records to those of Fig. 2 are given in Fig. 3 for one of the two fingers maintained in 14 per cent. oxygen during the period at 53° F. It will be seen that in this finger the incidence of ripening was delayed by the treatment given so that the fruit was still green on transfer to 68° F. Its

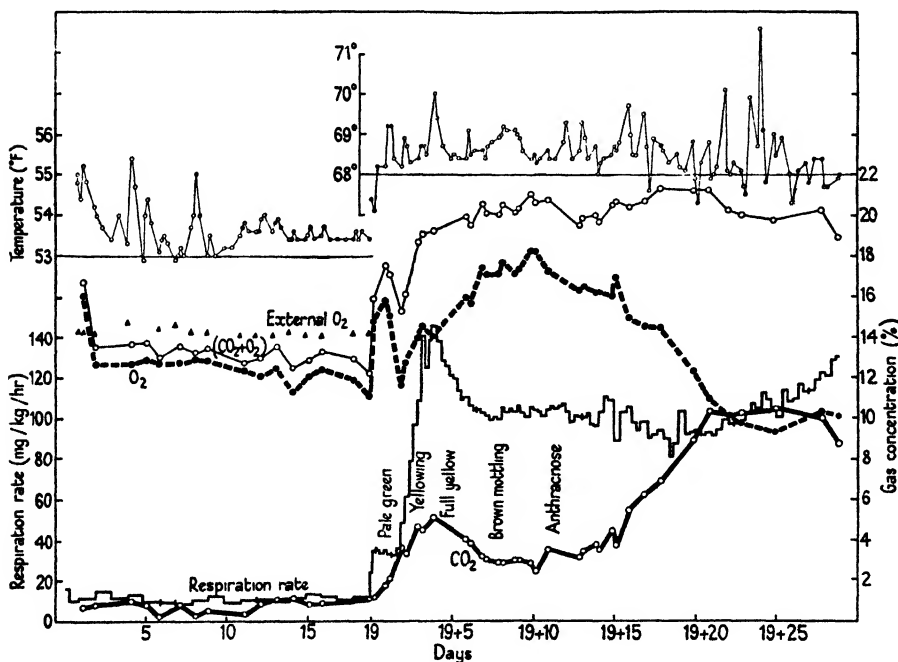


FIG. 3. Rate of respiration and internal concentrations of CO_2 , O_2 , and $\text{CO}_2 + \text{O}_2$ of a 'heavy $\frac{3}{4}$ -full' banana at 100 per cent. R.H. in 14 per cent. O_2 for 19 days at 53° F., followed by air (20.9 per cent. O_2) at 68° F. External O_2 concentrations at 53° F., black triangles. Air-temperature record shown.

subsequent ripening record, as given by internal gas concentrations and respiration rate, is comparable with that for fruit receiving a shorter period of storage at 53° F. and therefore not chilled (e.g. compare Figs. 12 and 13, Leonard and Wardlaw, 1941). The fall in internal oxygen concentration at the climacteric was not well marked. Additional data for this and its companion finger are given in Table III.

The rise in internal carbon dioxide concentration on transfer to 68° F. was closely followed by the rise with the climacteric in the finger of Fig. 3, whereas a brief level period occurred in the replicate finger.

A further delay in ripening was shown by the two fingers in still lower oxygen concentration at 53° F. (one cylinder of approximately 5.6 per cent. oxygen followed by one of 6.8 per cent.). The record for one finger is given in Fig. 4 and additional data are given in Table IV. The climacteric fall and

TABLE III

Weights, Respiration Data, and External Changes observed in Banana Fingers in 14 per cent. Oxygen at 53° F. followed by Air at 68° F.

Weight (gm.).	Loss (%)	Total days.	Mean pre-climacteric				Initiation of climacteric rise on basis of				Senescent rise in respiration rate.			
			Pulp/skin.		Int. conc.		Respiration rate (mg./kg./hr.)	(1) Internal CO ₂ rise and O ₂ fall.	(2) Respiration rate rise.	Full yellow skin.		Brown mottling.	Anthrax.	
Initial.	Final.		CO ₂ %	O ₂ %										
Finger of Fig. 3	179.15	162.15	9.48	19+29	2.03	0.8	12.6	11.5	19+0	19+2	19+5	19+8	19+11	19+21
Replicate.	169.24	157.37	7.02	19+22	2.32	1.0	12.2	12.6	19+4	19+3	19+8	19+10	19+12	19+21
							(falling)							

TABLE IV

Weights, Respiration Data, and External Changes observed in Banana Fingers in 5 and 7 per cent. Oxygen at 53° F. followed by Air at 68° F.

Weight (gm.).	Loss (%)	Total days.	Mean pre-climacteric			Initiation of climacteric rise on basis of			Senescent rise in respiration rate.			
			Initial.	Final.	Int. conc. CO ₂ %	Pulp/skin.	Respiration rate. (mg./kg./hr.)	(1) Internal CO ₂ rise and O ₂ fall.		(2) Respiration rate rise.		
					0.6	5.2	8.3					
					0.8	5.6	(un 5.6% O ₂)	19+6	19+7	19+10	19+14	?
							12.8					
							(in 6.8% O ₂)					
					0.6	(17.4)*	9.6	19+0	19+8	19+12	19+16	19+17
							(un 6.0% O ₂)					
Finger of Fig. 4	181.70	164.50	9.46	19+31	1.71							19+29
Replicate.	183.16	164.17	10.36	19+32	2.02							19+30

* The internal gas-sampling tube (Wardlaw and Leonard, 1939) to the replicate finger was apparently leaking, with a result that the internal oxygen estimations were high due to dilution with atmospheric oxygen. The subsequent behaviour of the internal gases of this finger at 68° F. was also abnormal, for the rise and fall in internal carbon dioxide concentration with accompanying fall and rise in oxygen occurred considerably in advance of the rise in respiration rate.

subsequent rise in internal oxygen concentration in Fig. 4 are more marked than in Figs. 2 or 3.

(b) *Respiration in 4 per cent. oxygen.*

A further series of experiments was made using cylinders of nitrogen from a local factory containing the minimum percentage of oxygen obtainable there. Four replicate fingers were supplied with gas from two cylinders, in pairs.

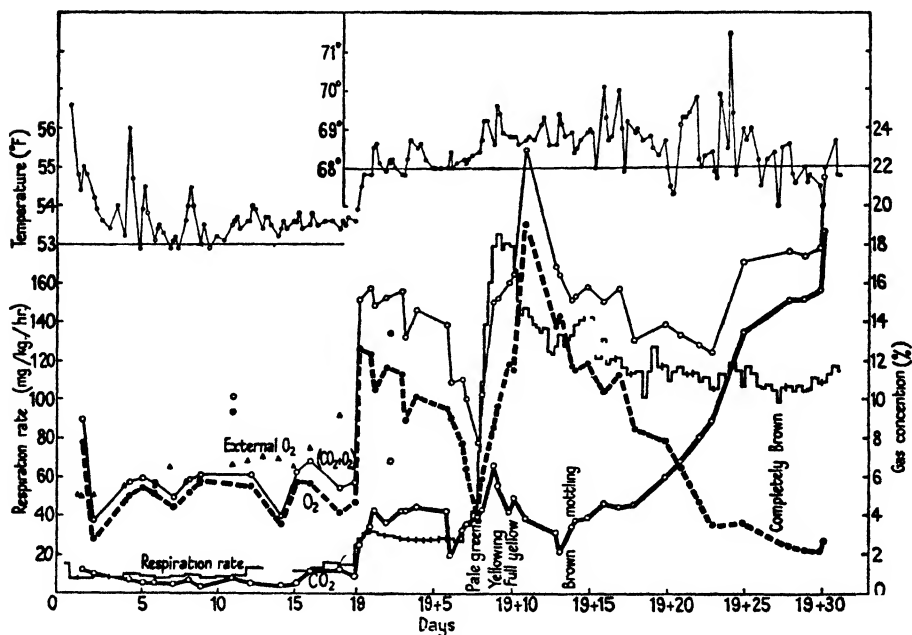


FIG. 4. Rate of respiration and internal concentrations of CO_2 , O_2 , and $\text{CO}_2 + \text{O}_2$ of a 'heavy $\frac{3}{4}$ -full' banana at 100 per cent. R.H. in 5.6 and 6.8 per cent. oxygen for 19 days at 53° F., followed by air (20.9 per cent. O_2) at 68° F. External O_2 concentrations at 53° F., black triangles. Air-temperature record shown.

The respiration record for one finger only in 4 per cent. oxygen is given in Fig. 7. The weights of the experimental bunch, hand, and fingers are given below:

Weight of intact bunch (8 hands)	21.50 kg.
Weight of second hand	3.03 "
Mean initial weight of upper row fingers of second hand	166.08 gm.
Initial weight of a fourth hand finger	160.47 "
Pulp/skin ratio	1.69 "

On the basis of weight of fingers the bunch was therefore 'heavy $\frac{3}{4}$ -full' with a rather high pulp/skin ratio. Its behaviour, however, was anomalous and of the type discussed previously (Wardlaw and Leonard, 1940, pp. 287-9; Leonard and Wardlaw, 1941, pp. 394-404). (i) The preclimacteric period was considerably longer than would have been anticipated for fruit of this grade; (ii) the decline in oxygen concentration was, in six out of eight fingers,

less than normal; and (iii) difficulty was found in determining the incidence of the 'sprung' condition.

Fig. 5 gives the record of internal gas concentrations in one of the fingers

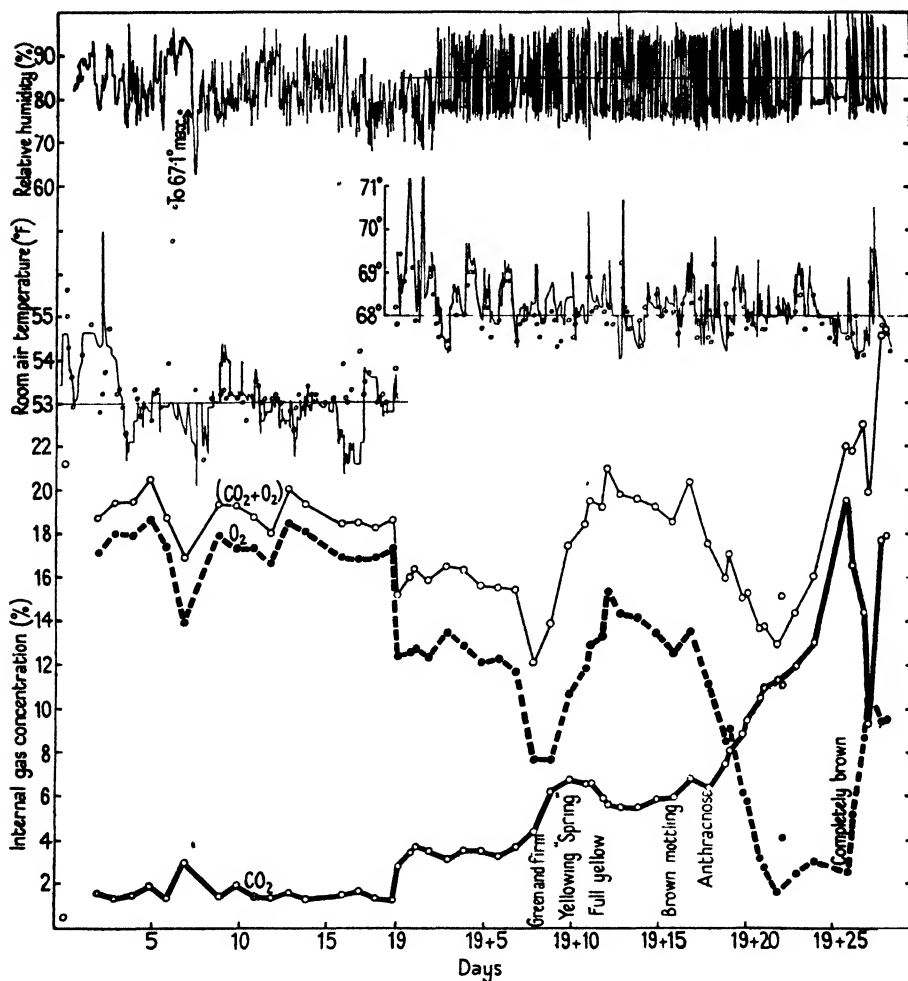


FIG. 5. Internal concentrations of CO_2 , O_2 , and $\text{CO}_2 + \text{O}_2$ of a 'heavy $\frac{3}{4}$ -full' banana in air for 19 days at 53°F . and about 80 per cent. R.H. followed by 68°F . and 85 per cent. R.H. Thermograph and point records of temperature and hygrograph record shown.

exposed to the room atmosphere.¹ It will be seen that the record agrees very closely with that for fruit at 53°F . for the normal period (14 days) and then transferred to 68°F . (Leonard and Wardlaw, 1941, Fig. 5). The replicate finger, however, gave an anomalous record. At the end of the experiment the internal gas-sampling tube was found to be plugged with pulp tissue. Only

¹ A temporary breakdown in the refrigeration plant on the 6th to 7th day caused a considerable rise in temperature and was accompanied by a rise in internal carbon dioxide concentration and fall in oxygen.

TABLE V

Weights, Respiration Data, and External Changes observed in Banana Fingers in Air at 53° F. followed by 68° F.

	Mean pre-climacteric				Pulp/ skin.	Total days.	Loss (%).	Days to					
	at 53° F.		at 68° F.					Initiation of CO ₂ rise and O ₂ fall.	Full yellow skn.	Brown mott- ling.	Anthraxnose.		
	Internal concentration												
	CO ₂ %	O ₂ %	CO ₂ %	O ₂ %									
Finger of Fig. 5	164.23	120.37	26.71	19+28	4.43	1.4	17.4	3.4	12.6	19+6	19+12	19+16	19+18
Replicate .	168.87	122.82	27.27	19+26	4.11	(1.0	19.4	see text)		(4, see text)	19+10	19+14	19+16

TABLE VI

Weights, Respiration Data, and External Changes observed in Banana Fingers in Air at 53° F. followed by 68° F.

	Weight (gm.)		Loss (%).	Total days.	Pulp/ skin.	Mean pre-climacteric						Days to						
	Initial.	Final.				at 53° F.		at 68° F.		Respiration (mg./kg./hr.)		Int. conc. CO ₂ % O ₂ %	Initiation of climacteric rise on basis of		Full yellow skin.	Brown mott- lung.	An- thrax- nose.	Senescent rise in respiration rate.
						Initial.	Final.	CO ₂ %	O ₂ %	Rate	Int. conc.		(1) Internal CO ₂ rise and O ₂ fall.	(2) Respira- tion rate				
Finger of Fig. 6	177.19	168.29	5.02	19+27	2.61	1.4	17.6	12.4	3.8	14.2	19+4	19+7	19+13	19+17	19+18	19+23		
Replicate .	164.06	155.97	4.93	19+29	2.57	1.2	18.0	16.6	Slow rise	Slow fall	19+0	19+8	19+14	19+17	19+18	19+26		

small samples of gas had been obtained with consequent loss of accuracy. The internal carbon dioxide concentrations rose fairly steadily throughout the period at 53° F., then rapidly to a peak on transfer to 68° F. with a subsequent sustained high level: a fall in oxygen concentration, slow at 53° F. and rapid on transfer to 68° F. to a steady low level, accompanied this.

As based on colour changes in the skin, the behaviour of this replicate finger was similar to that of Fig. 5. Additional data are given in Table V.

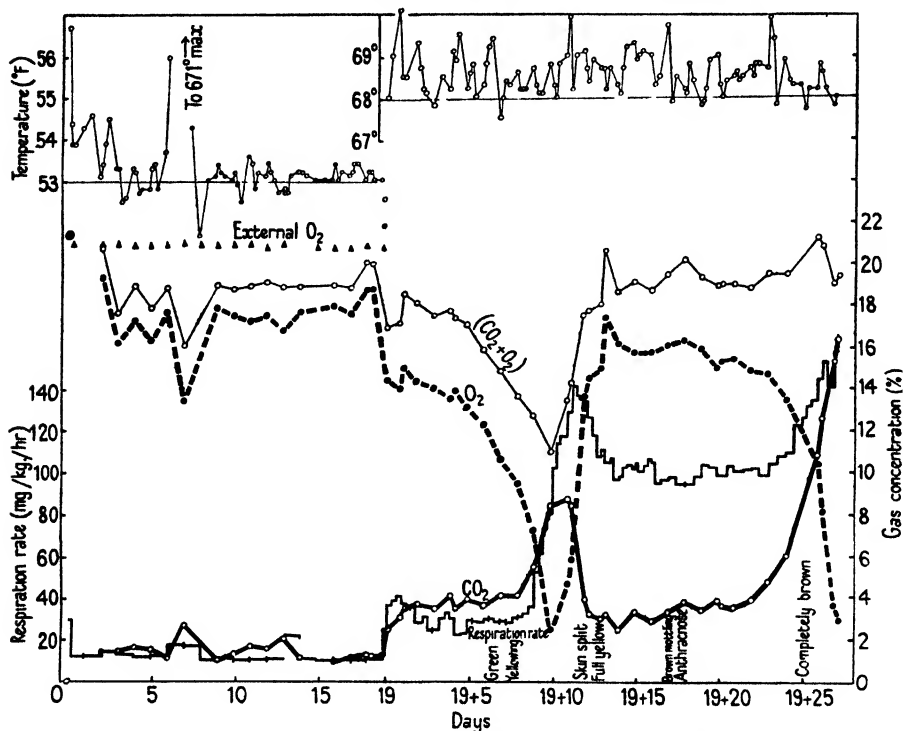


FIG. 6. Rate of respiration and internal concentrations of CO_2 , O_2 , and $\text{CO}_2 + \text{O}_2$ of a 'heavy $\frac{3}{4}$ -full' banana in air (20.9 per cent. O_2) at 100 per cent R.H. for 19 days at 53° F., followed by 68° F. External O_2 concentrations at 53° F., black triangles. Air-temperature record shown.

The corresponding record for a finger in a respiration chamber supplied with air is given in Fig. 6. The general trend of internal gas concentrations is very similar except that a somewhat more pronounced peak and trough of carbon dioxide and oxygen concentrations respectively occurred at the climacteric. The respiration rate curve was in all respects similar to that of a fruit removed to 68° F. after a period of only 14 days at 53° F. (cf. Leonard and Wardlaw, 1941, Fig. 1). Table VI gives additional data.

It was noted that in these fingers the appearance of anthracnose followed very closely that of brown mottling of the skin, also that shortly after the climacteric peak the skin split along one of the sutures. This did not occur in the fingers (Fig. 5 and replicate, at the lower humidity of the room atmo-

sphere. This phenomenon has been noticed previously (Leonard and Wardlaw, 1941).

A single, typical record only is given of the four fingers in 3 to 4 per cent. oxygen at 53° F., Fig. 7. The period of steady gas concentrations and respiration rate at 68° F. is seen to be considerably extended beyond those of the fingers of Figs. 5 and 6. The eventual climacteric rise and fall, however, were

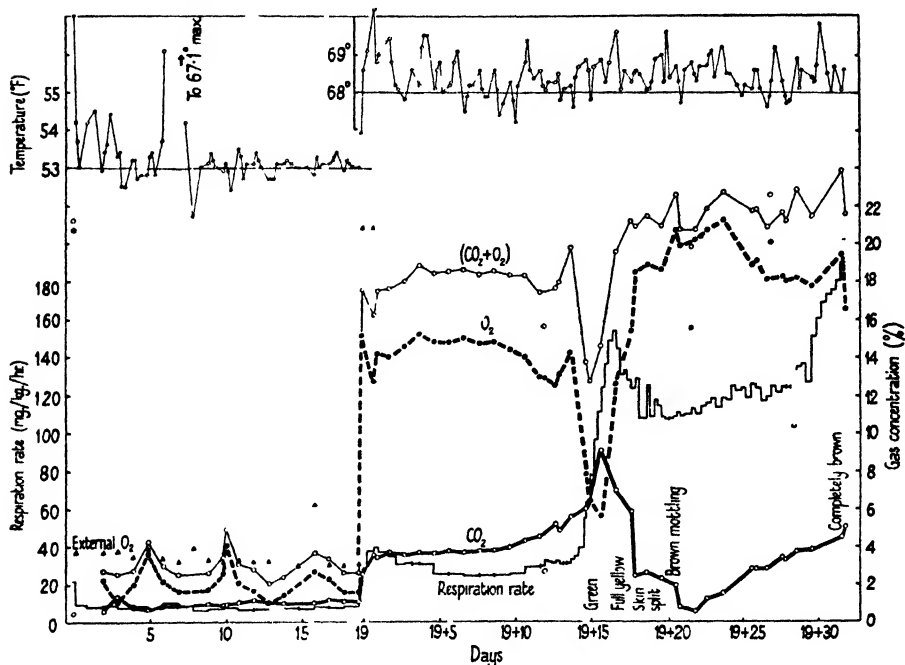


FIG. 7. Rate of respiration and internal concentrations of CO_2 , O_2 , and $\text{CO}_2 + \text{O}_2$ of a 'heavy $\frac{3}{4}$ -full' banana at 100 per cent. R.H. in 3 to 4 per cent. oxygen for 19 days at 53° F., followed by air (20.9 per cent. O_2) at 68° F. External O_2 concentrations at 53° F., black triangles. Air temperature record shown.

of a normal type and the appearance of the fingers was comparable with those of Fig. 6. Three of the four fingers showed skin splitting soon after the climacteric peak. Additional data for the four fingers are tabulated in Table VII.

(c) Respiration in nitrogen containing less than 1 per cent. oxygen.

A final experiment with reduced oxygen concentrations was made using cylinders of imported nitrogen, containing the normal low percentage of oxygen (about 0.5 per cent.) of commercial nitrogen. Unfortunately the supply gave out before the completion of the period at 53° F. and during the last 3 days gas was supplied from locally produced nitrogen (containing 4 per cent. oxygen). Considerable fluctuations of temperature at 53° F. and at 68° F. and other mishaps occurred during this experiment and difficulties were also experienced with leaks in the respiration system.

TABLE VII

Weights, Respiration Data, and External Changes observed in Banana Fingers in 4 per cent. Oxygen at 53° F. followed by Air at 68° F.

	Days to																					
	Mean pre-climacteric										Initiation of climacteric rise on basis of											
	at 53° F.					at 68° F.																
	Weight (gm.).		Loss (%)	Total days.	Pulp, skm.	Int. conc.		Respiration rate		Int. conc.		Respiration rate		(1) Internal CO ₂ rise and O ₂ fall.		(2) Respiration rate rise.		Full yellow skin.	Brown mottling.	Anthrax-nose.	Senescent rise in respiration rate.	
Initial.	Final.				CO ₂ %	O ₂ %	CO ₂ %	O ₂ %	(mg./kg./hr.)	(mg./kg./hr.)	CO ₂ %	O ₂ %	(mg./kg./hr.)	(mg./kg./hr.)								
Finger of Fig. 7																						
	161.34	151.84	5.89	19+32	2.20	0.9	2.0	7.7	26	19+9	19+14	19+18	19+21	19+26*	19+28							
(variable)																						
Replicate 1	161.10	152.32	5.45	19+33	2.23	1.0	2.0	8.2	22	19+8	19+14	19+18	19+21	19+23	19+28							
Replicate 2	160.77	148.65	7.54	19+36	1.86	0.8	2.4	8.4	20	19+14	19+14	19+19	19+23	19+26*	19+27							
Replicate 3	171.04	159.42	6.80	19+34	2.03	0.8	2.9	13.7	31	19+13†	19+13	19+19	19+22	19+24*	19+28							
to 1.5 to 2.0 (variable)																						

* First incidence not observed.

† No drop in oxygen concentrations, slight rise in carbon dioxide.

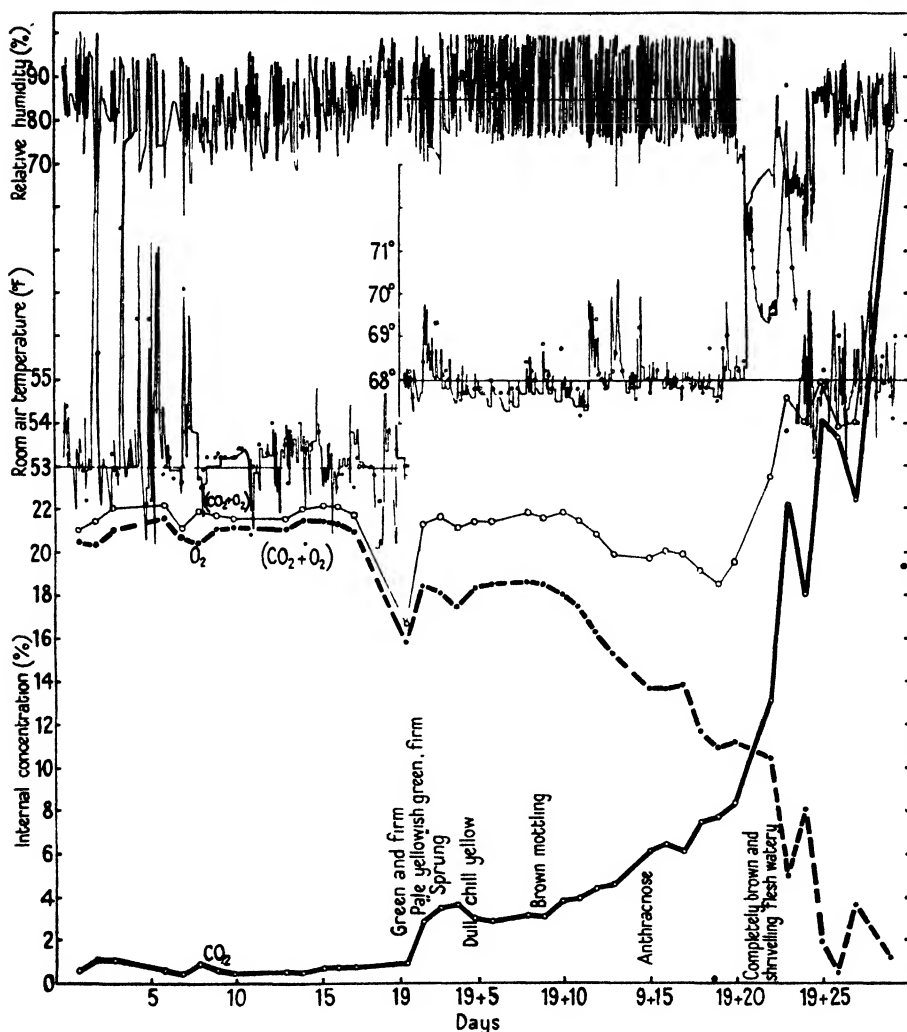


FIG. 8. Internal concentrations of CO_2 , O_2 , and $\text{CO}_2 + \text{O}_2$ of a 'heavy $\frac{3}{4}$ -full' banana in air at 53° F. and 70–90 per cent. R.H. for 19 days, followed by 68° F. and 85 per cent. R.H. Thermograph and point records of temperature and hygrograph record shown.

Four replicate fingers were again supplied with these low concentrations of oxygen: only one record is given. The weights of the experimental bunch, hand, and fingers are shown below:

Weight of intact bunch (10 hands)	24.2 kg.
Weight of second hand	3.36 „
Mean initial weight of upper row fingers of second hand	157.16 gm.
Initial weight of one upper row finger	148.16 „
Initial pulp/skin weight ratio	1.53 „

The weight and the pulp/skin ratio grade the fruit as 'heavy $\frac{3}{4}$ -full'.

Fig. 8 gives the record of internal gas concentrations for a finger in air throughout; it is similar to Fig. 1, i.e. for a banana whose climacteric rise

began at 53° F., except that the incidence of the climacteric is somewhat later, the climacteric fall in internal oxygen and rise in carbon dioxide concentrations being superimposed on the corresponding effects produced by the rise in temperature.

Additional data for this finger and its replicate are shown in Table VIII.

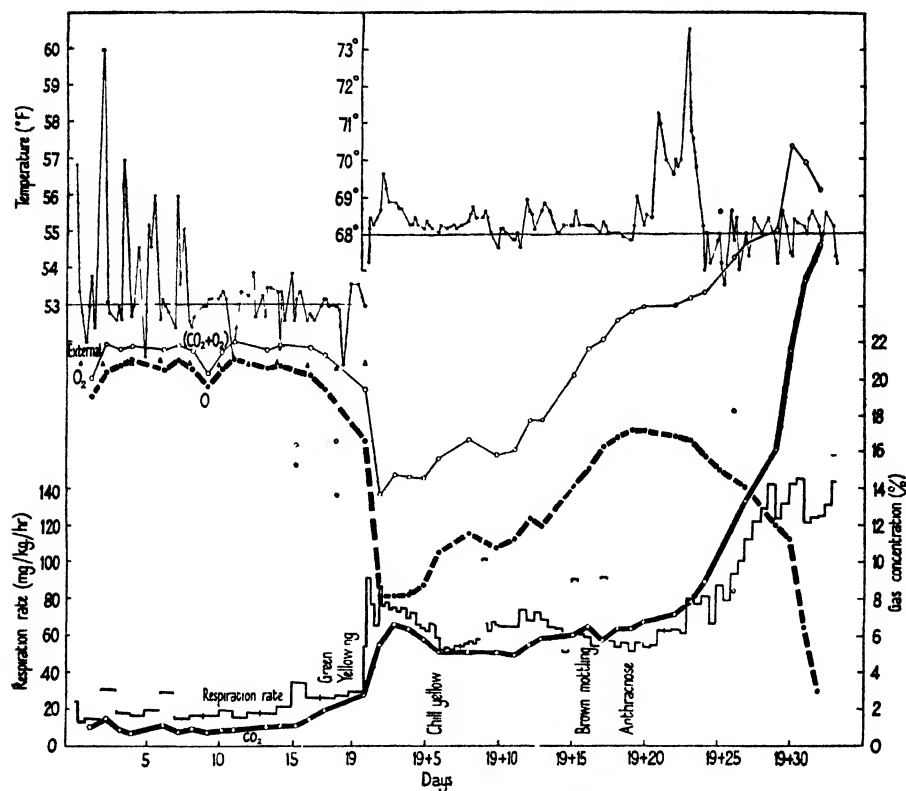


FIG. 9. Rate of respiration and internal concentrations of CO_2 , O_2 , and $\text{CO}_2 + \text{O}_2$ of a 'heavy $\frac{3}{4}$ -full' banana in air (20.9 per cent. O_2) at 100 per cent RH for 19 days at 53° F., followed by 68° F. External O_2 concentrations at 53° F., black triangles. Air temperature record shown.

The corresponding record for a finger in a saturated atmosphere is given in Fig. 9 together with its respiration rate. Apart from a later incidence of the climacteric these curves are similar to those of Fig. 2. Table IX gives additional data for this finger and its replicate.

The record for a single finger of the four supplied with nitrogen containing less than 1 per cent. oxygen during the period at 53° F. is given in Fig. 10. The records for two of the fingers in this experiment were inaccurate in respect of respiration rate or internal gas concentrations owing to leaks either in the respiration chamber and its connexions or at the point of entry of the gas-sampling tube. Steady respiration rates, after the initial fall and before supplying 4 per cent. oxygen, were found for the two fingers not affected by

TABLE VIII

Weights, Respiration Data, and External Changes observed in Banana Fingers in Air at 53° F. followed by 68° F.

	Weight (gm.).		Loss (%).	Total days.	Pulp/ skin.	Mean pre-climac- teric at 53° F.		Days to			
	Initial.	Final.				Int. conc.	CO ₂ %	Initiation of cli- matic rise and O ₂ fall.	Full yellow skin.	Brown mott- ling.	Anthraco- nose.
Finger of Fig. 8	161.04	96.21	21.40	19+29	3.50	0.7	21.0	17 to 19 (?)	19+5	19+9	19+15
Replicate	154.38	109.21	29.26	19+23	3.14	1.0	20.4	19+0	19+5	19+10	19+15

TABLE IX

Weights, Respiration Data, and External Changes observed in Banana Fingers in Air at 53° F. followed by 68° F.

	Weight (gm.).		Loss (%).	Total days.	Pulp/ skin.	Mean pre-climac- teric at 53° F.		Days to			
	Initial.	Final.				Int. conc.	Respira- tion rate. (mg./kg./hr.)	Initiation of climac- teric rise on basis of	Full yellow skin.	Brown mott- ling.	Senescent rise in respira- tion rate.
								(1) Internal CO ₂ rise and O ₂ fall.			
								(2) Respira- tion rate rise.			
Finger of Fig. 9	149.72	136.37	8.52	19+32	1.71*	0.9	20.6	15	14	19+6	19+21
Replicate	163.77	141.11	13.83	19+38	1.43*	0.7	20.8	14	14	19+5	19+19

* The total storage life of these fingers was somewhat longer than those of the preceding ones (Section III (a) and (b)); and the pulp/skin ratios are lower owing to greater loss of water from the pulp, the skin having reached a dried-up, leathery condition.

leaks. Fig. 10 resembles Figs. 3, 4, 6, and 7 in showing a definite preclimacteric level of respiration at 68° F., i.e. the climacteric rise is delayed until some time after transfer to this temperature, and a subsequent well-marked rise and fall is shown.

Additional data are given in Table X for the four fingers.

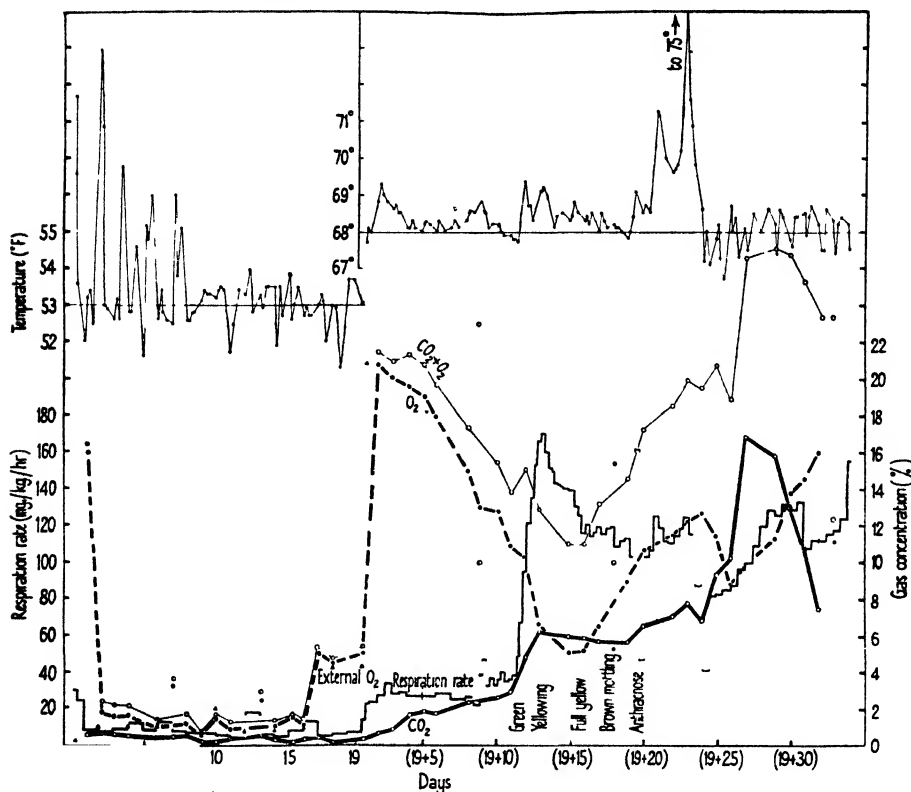


FIG. 10. Rate of respiration and internal concentrations of CO_2 , O_2 , and $\text{CO}_2 + \text{O}_2$ of a 'heavy $\frac{3}{4}$ -full' banana at 100 per cent. R.H. in nitrogen containing less than 1 per cent. oxygen for 19 days at 53° F., followed by air (20.9 per cent. O_2) at 68° F. External O_2 concentrations at 53° F., black triangles. Air-temperature record shown.

The final ripe colour of the fingers previously in the low oxygen atmosphere was quite normal yellow, whereas the extent of 'chilling' in the controls in air throughout was such that dull 'chill' yellow skin occurred and not a bright yellow such as results from only slight chilling.

IV. RESPIRATION IN OXYGEN CONCENTRATIONS GREATER THAN IN AIR

Respiration in 24 per cent. and 55 per cent. Oxygen

Two experiments were conducted comparing these concentrations with air (20.9 per cent. oxygen) as control.

(i) Fingers from the third hand of a somewhat more than 'heavy $\frac{3}{4}$ -full' bunch with a somewhat abnormally high pulp/skin ratio were used.

TABLE X
Weights, Respiration Data, and External Changes observed in Banana Fingers in Nitrogen containing less than 1 per cent. Oxygen at 53° F., followed by Air at 68° F.

	Mean pre-climacteric										Initiation of climacteric rise on basis of		Days to			
	at 53° F.					at 68° F.					(1) CO ₂ rise and O ₂ fall. (2) Rise in re- spirational rate.		Full yellow skin.	Brown mottling.	Anthrax-nose.	Senescent rise in respiration rate.
	Int. conc.		Respiration rate (mg./kg./hr.)	Int. conc.		Respiration rate (mg./kg./hr.)										
	Weight (gm.). Initial. Final.	Loss (%)		Total days.	Pulp/skin.		CO ₂ %	O ₂ %	CO ₂ %	O ₂ %						
Finger of Fig. 10	156.85	142.93	8.87	19+34	1.60	0.4	1.2	6.4	1.8 fall- (rising) ing	28	19+8 (CO ₂ rise)	19+9	19+16	19+18	19+20	19+25
Replicate 1	161.50	140.93	12.74	19+38	1.50	0.4	0.8	6.5	1.8	19.6	24	19+11	19+15	19+18	19+19	19+26
Replicate 2	159.73	145.33	9.02	19+29	1.88	0.4	6.0	11.8	variable	26		19+6	19+11	19+13	19+15	19+25
Replicate 3	159.30	142.61	10.47	19+31	1.81	0.5	1.0	11.0	0.9	20.0	26	19+8	19+15	19+17	19+19	19+26

All the fingers of the hand used for respiration measurement showed ripening changes immediately following cutting without any differences ascribable to the oxygen content of the environmental air. No details of this experiment are therefore given in this paper, which is concerned chiefly with the respiration of green, unripe bananas at 53° F. in different oxygen concentrations.

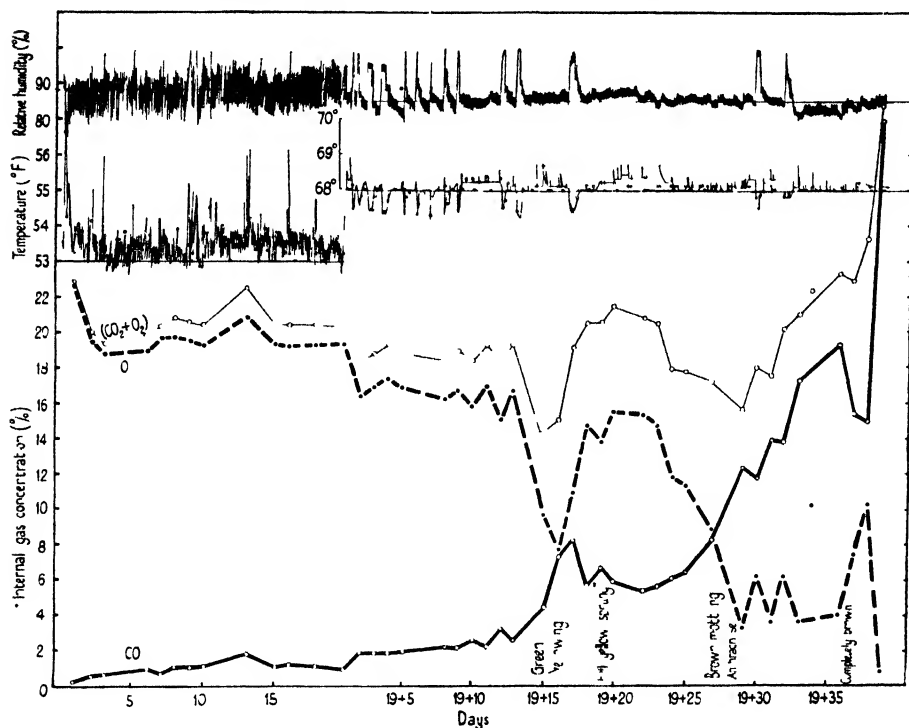


FIG. 11. Internal concentrations of CO_2 , O_2 , and $\text{CO}_2 + \text{O}_2$ of a 'heavy $\frac{3}{4}$ -full' banana in air at 53° F. and 83 per cent. R.H. for 19 days, followed by 68° F. and 85 per cent. R.H. Thermograph and point records of temperature and hygrograph record shown

(ii) In a second experiment fruits from a somewhat less mature bunch were taken, the data of which are given below:

Weight of intact bunch (10 hands)	27.0 kg.
Weight of third hand	3.24 "
Mean initial weight of upper row fingers of third hand	155.75 gm
Weight of one finger from this row and hand	157.62 "
Initial pulp/skin ratio of this finger	1.57 "

On the basis of weight and pulp/skin weight ratio this bunch was therefore 'heavy $\frac{3}{4}$ -full'. It showed the anomalous behaviour found in the fruit of Section III (b), namely, a prolonged pre-climacteric period, and in addition somewhat irregular ripening of individual fingers and atypical sequence of ripening of the separate hands. This is partly a reflection of the long time at 68° F. (after 19 days at 53° F.), the uneven incidence of stem-end rotting giving irregular ripening stimulus.

Fig. 11 gives the internal gas concentrations of one of two fingers exposed to the room atmosphere, and the record of the temperature and humidity (53° F. and 88 per cent. R.H. followed by 68° F. and 85 per cent. R.H.). A slightly higher mean relative humidity of the room air obtained at 53° F. than in earlier experiments, due to an alteration in the refrigeration control mechanism. The replicate finger showed evidence of a fungal infection at the point of insertion of the gas sampling tube after 10 days at 53° F., a steady

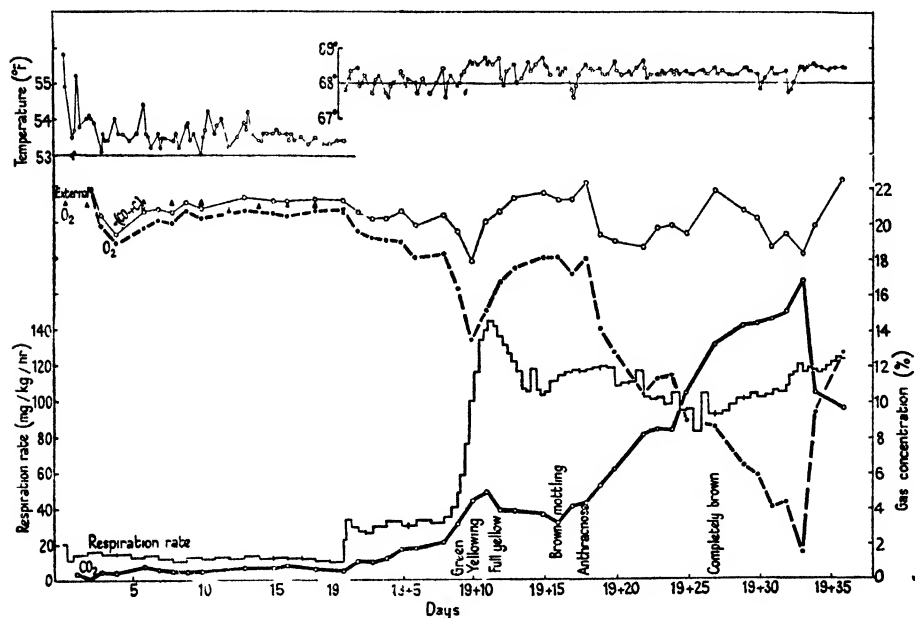


FIG. 12. Rate of respiration and internal concentrations of CO_2 , O_2 , and $\text{CO}_2 + \text{O}_2$ of a 'heavy $\frac{3}{4}$ -full' banana in air (20.9 per cent. O_2) at 100 per cent. R.H. for 19 days at 53° F., followed by 68° F. External O_2 concentrations at 53° F., black triangles. Air temperature record shown.

rise in the internal carbon dioxide and fall in oxygen concentration occurring. Rapid fungal wastage ensued on subsequent transfer to 68° F. The finger whose internal gas concentration record is given in Fig. 11 showed steady values of carbon dioxide and oxygen during the entire period of 19 days at 53° F. This was followed by a rise in internal carbon dioxide to a steady value at 68° F. for 10 days accompanied by a steady, reduced oxygen concentration. Subsequently the climacteric rise and fall and final senescent rise in carbon dioxide concentration took place, with climacteric fall and rise in oxygen concentration and final senescent fall to low value. Additional data are shown in Table XI.

The records of respiration rate and internal gas concentrations of a finger in saturated air are given in Fig. 12. These are typical of a banana which did not sustain 'chilling' injury during the period at 53° F. and subsequently ripened in air at 68° F. giving a normal climacteric. The fall in internal

TABLE XI

Weights, Respiration Data, and External Changes observed in Banana Fingers in Air at 53° F. followed by 68° F.

	Weight (gm.).		Loss (%).	Total days.	Pulp/ skin.	Mean int. conc. at 53° F.		Mean pre-climacteric at 68° F. int. conc.		Days to				
	Initial.	Final.				CO ₂ %	O ₂ %	CO ₂ %	O ₂ %	Initiation of climacteric by CO ₂ rise and O ₂ fall.	Full yellow skin.	Brown mott- ling.	An- thrac- nose.	Senescent rise in CO ₂ , fall in O ₂ .
Finger of Fig. 11	155.54	97.40	37.40	19+39	4.94	1.0	19.2	2.0	16.4	19+13	19+19	19+27	19+28	19+22
Replicate	158.80	109.05	31.32	19+22	1.73	1.0*	19.2*	steady rise	steady fall	19+1	19+7	19+15	—	—

* During first 10 days, then CO₂ rising O₂ falling, see text.

TABLE XII

Weights, Respiration Data, and External Changes observed in Banana Fingers in Air at 53° F. followed by 68° F.

	Weight (gm.).		Loss (%).	Total days.	Pulp/ skin.	Mean pre-climacteric at 53° F.			Mean pre-climacteric at 68° F.			Days to				
						Int. conc. CO ₂ %	O ₂ %	Respiration (mg./kg./hr.)	Int. conc. CO ₂ %	O ₂ %	Respiration (mg./kg./hr.)	Initiation of climacteric on basis of (1) CO ₂ rise and O ₂ fall. (2) Rise in rate of respiration	Full yellow skin.	Brown mottling.	Anthrax-nose.	Senescent rise in respiration rate.
	Initial.	Final.														
Finger of Fig. 12	146.80	126.05	14.14	19+36	2.34	0.6	20.6	13.4	2.0	19.2	32	19+8	19+8	19+12	19+16	19+27
Replicate	158.07	139.66	11.64	19+32	2.55	0.8	19.8	12.4	2.4	15.0	24	19+12	19+11	19+16	19+22	19+30
									(slow rise)	(slow fall)						
									(slow rise)	(slow fall)						

oxygen concentration during the climacteric was, however, not as marked as in the fruit of Figs. 2, 6, and 9. Additional data are shown in Table XII.

The replicate finger gave similar records of respiration and internal gas changes.

Fig. 13 gives the record of a finger supplied with 24 per cent. oxygen¹ whilst at 53° F. A slowly declining respiration rate at 53° F. was accompanied by a steady internal carbon dioxide concentration. On transfer to air at 68° F.

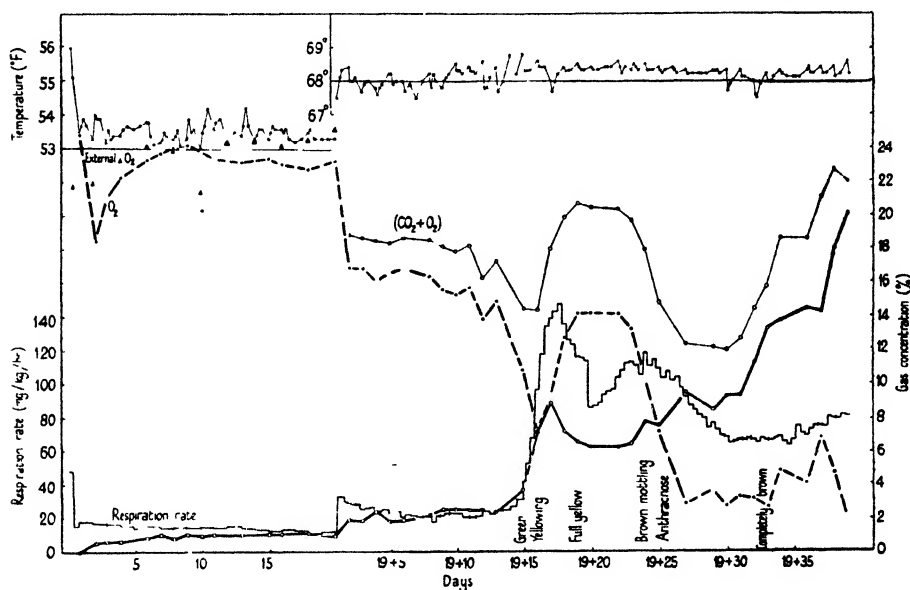


FIG. 13. Rate of respiration and internal concentrations of CO_2 , O_2 , and $\text{CO}_2 + \text{O}_2$ of a 'heavy $\frac{3}{4}$ -full' banana at 100 per cent. R.H. in 24 per cent. oxygen at 53° F. for 19 days, followed by air (20.9 per cent. O_2) at 68° F. External O_2 concentrations at 53° F., black triangles. Air temperature record shown.

a normal climacteric was obtained. Table XIII shows the additional data for these two fingers.

No difference is observable between these fingers in 24 per cent. oxygen and those in air either in the rate of respiration or the internal carbon dioxide concentration at 53° F., nor is there a consistent difference in the length of time at 68° F. prior to the climacteric rise.

In 55 to 56 per cent. oxygen at 53° F. two fingers showed slight differences from those above in air and in 24 per cent. oxygen. The data are given in Fig. 14 and in Table XIV.

The finger whose record is given in Fig. 14 showed neither sustained low internal oxygen nor high carbon dioxide concentrations during late senescence, whereas the replicate did. Such variations have been noted previously (Wardlaw and Leonard, 1940, pp. 280 and 286-7; Leonard and Wardlaw, 1941,

¹ Owing to improper adjustment of outlet pressure from the cylinder of gas the required concentration of oxygen was not reached until the sixth day of storage.

pp. 403-4). Differences were also seen in the pre-climacteric respiration rate at 68° F. but none in the time of incidence of the climacteric. All fingers in air, 24 per cent. and 55 per cent. oxygen at 53° F., ripened to a good yellow colour at 68° F. It is possible that oxygen concentrations higher than that in air stimulate ripening changes when supplied to green bananas shortly before the unstimulated climacteric and/or at higher temperature.

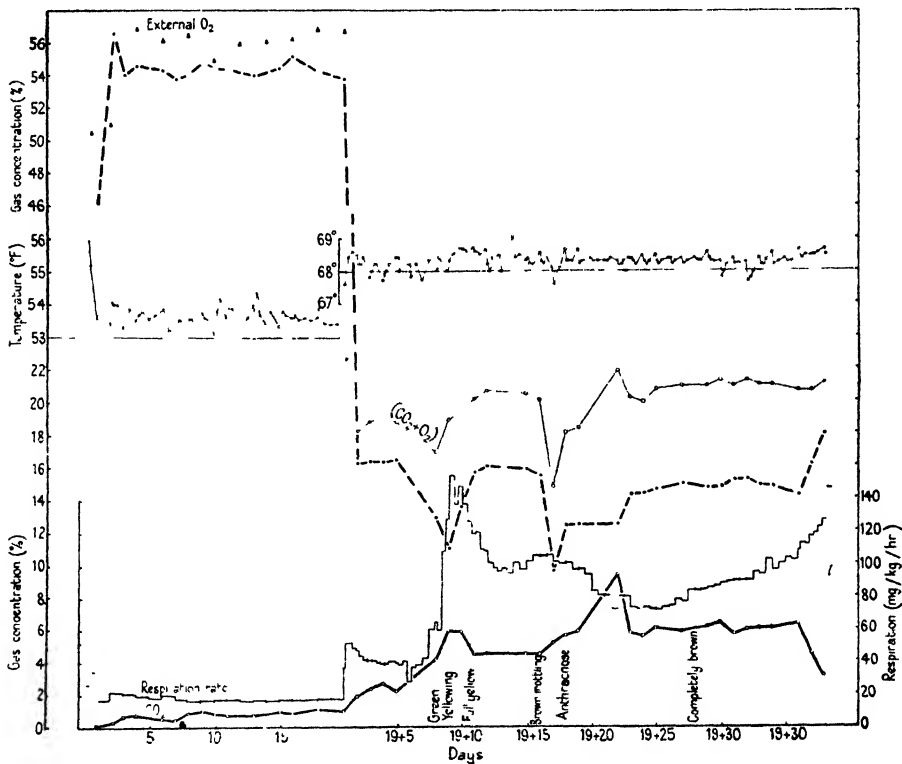


FIG. 14. Rate of respiration and internal concentrations of CO_2 , O_2 , and $\text{CO}_2 + \text{O}_2$ of a 'heavy $\frac{3}{4}$ -full' banana at 100 per cent. R.H. in 55 per cent. oxygen at 53° F. for 19 days, followed by air (20.9 per cent. O_2) at 68° F. External O_2 concentrations at 53° F., black triangles. Air temperature record shown.

V. COMPARISON OF RESPIRATION DATA IN DIFFERENT OXYGEN CONCENTRATIONS

The data for the different oxygen concentrations during the period at 53° F. and in a saturated atmosphere are assembled in Fig. 15.

From the curve showing the relationship of respiration rate and external oxygen concentrations it appears that the concentration in air is critical, a fairly steep fall in respiration rate occurring below this value. Increase in oxygen concentration above that in air produces only a slight increase in respiration rate in green bananas. (The region of maximum inflexion cannot be sharply determined from the data presented.) Respiration in oxygen-free

TABLE XIII

Weights, Respiration Data, and External Changes observed in Banana Fingers in 24 per cent. Oxygen at 53° F. followed by Air at 68° F.

	Mean pre-climacteric										Initiation of climacteric rise on basis of				Days to			
	at 53° F.				at 68° F.				(1) CO ₂ rise and O ₂ fall.									
	Int conc		Respiration		Int conc		Respiration		Int conc		Respiration							
	CO ₂ %	O ₂ %	Rate (mg./kg./hr.)	Rate (mg./kg./hr.)	CO ₂ %	O ₂ %	Rate (mg./kg./hr.)	Rate (mg./kg./hr.)	CO ₂ %	O ₂ %	Rate (mg./kg./hr.)	Rate (mg./kg./hr.)	(2) Rise in respiration rate.	Full yellow skin.	Brown mottling.	Anthrax-nose.	Senescent rise in respiration rate.	
Finger of Fig. 13	155.11	134.84	13.07	1.0	23.2	14.4	2.2	15	22	19+13	19+13	19+13	19+13	19+19	19+24	19+25	19+35	
Replicate	157.43	134.37	14.65	0.7	23.0	13.6	1.8	16	28	19+5	19+7	19+13	19+17	19+19	19+25	19+35	19+46	
						(falling)	(falling)	(falling)										
						(falling)(rising)	(falling)											

TABLE XIV

Weights, Respiration Data, and External Changes observed in Banana Fingers in 55 to 56 per cent. Oxygen at 53° F. followed by Air at 68° F.

	Days to										
	Mean pre-climacteric						Initiation of climacteric rise on basis of				
	at 53° F.			at 68° F.							
	Respiration			Respiration			(1) CO ₂ rise and O ₂ fall.				
	Int conc.	CO ₂ %	O ₂ %	Int conc.	CO ₂ %	O ₂ %	(2) Rise in respiration rate.	Full yellow skin.	Brown mottling.	Anthrax-nose.	Senescent rise in respiration rate.
Pulp, skin	Total days.	Loss (%)	Weight (gm.).	Initial	Final.						

nitrogen is apparently somewhat less than half that in air (6 mg./kg./hr. compared with a mean value 14.2 mg./kg./hr.).

The small differences between the internal carbon dioxide concentrations at different external oxygen concentrations are insufficient to establish a trend, and a fairly steady value of 1.0 per cent. obtains except at very low oxygen

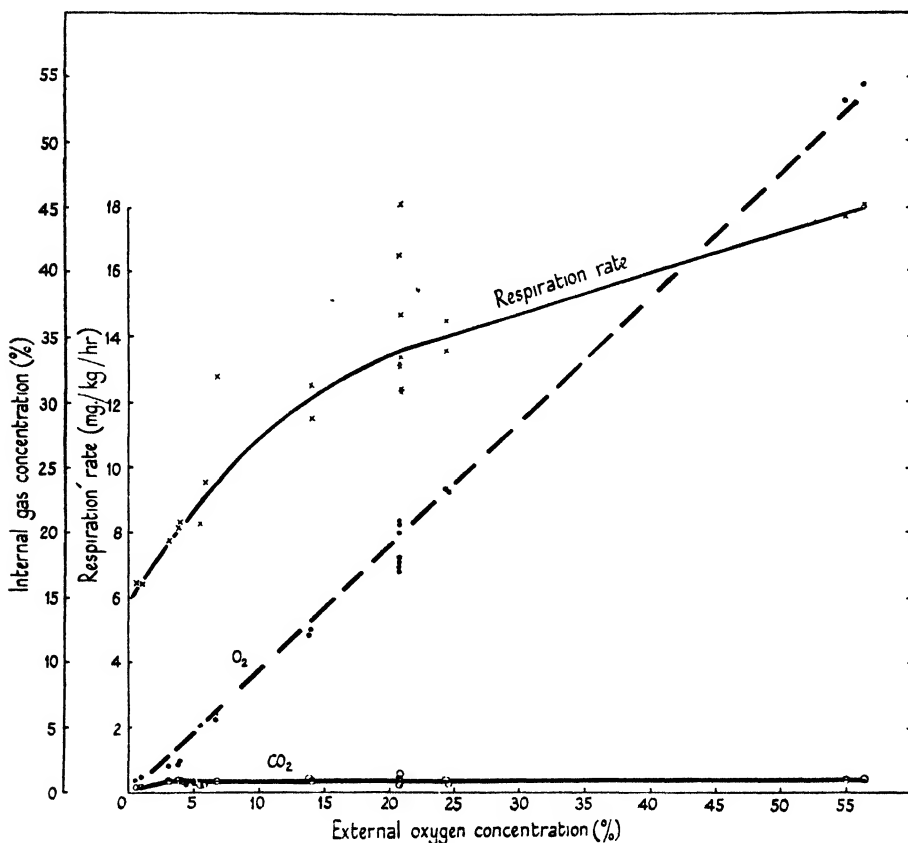


FIG. 15. Mean rate of respiration (crosses) and internal concentrations of CO_2 (white circles) and O_2 (black circles) of 'heavy $\frac{3}{4}$ -full' bananas during 19 days at 53°F . and 100 per cent. R.H. in different O_2 concentrations.

concentration. This value was earlier found to exist in bananas in air at 53°F . (Leonard and Wardlaw, 1941). The internal oxygen concentration shows a linear relationship with the outer oxygen concentration over the range used.

Since fingers from different bunches were used in the series of experiments no conclusions can be drawn as to the comparative extension of the storage period at 53°F . produced by reduction of external oxygen concentration. The data are assembled below. The range in respiration rate (12.4–18.1 mg./kg./hr., mean 14.2) of fingers in air at 53°F . shows no apparent relationship with initial weight of finger nor with initial pulp/skin weight ratio (of

adjacent fingers) but the present data cover an insufficient range of grade of fruit. No hypothesis can, as yet, be put forward to account for the considerable differences found in the time of onset of ripening changes of bunches of closely comparable grades subjected to the same period at 53° F. in air, with consequent chilling or the avoidance thereof. This individualism of bunches is presumably the result of differences in physiological maturity at harvesting resulting from different environmental conditions during growth (Wardlaw, Leonard, and Barnell, 1939; Wardlaw and Leonard, 1940).

TABLE XV

Collected Data showing Effect of Different Oxygen Concentrations at 53° F. on Time of Occurrence of Ripening Changes

	External O ₂ conc. (%).	Days		
		to initiation of cli- matic (on basis of respiration rate rise). Mean	Postponement due to different O ₂ atmosphere.	'Eating ripe' period (full yellow to anthracnose). Mean
III (a)	20.9 (air)	$\left. \begin{matrix} 12 \\ 15 \end{matrix} \right\} 13.5$	—	$\left. \begin{matrix} 6 \\ 7 \end{matrix} \right\} 6.5$
	14	$\left. \begin{matrix} 19+2 \\ 19+3 \end{matrix} \right\} 19+2.5$	5.5+2.5	$\left. \begin{matrix} 6 \\ 4 \end{matrix} \right\} 5$
	6	$\left. \begin{matrix} 19+7 \\ 19+8 \end{matrix} \right\} 19+7.5$	5.5+7.5	$\left. \begin{matrix} (?)5 \\ 5 \end{matrix} \right\} 5$
III (b)	20.9 (air)	$\left. \begin{matrix} 19+7 \\ 19+8 \end{matrix} \right\} 19+7.5$	—	$\left. \begin{matrix} 5 \\ 4 \end{matrix} \right\} 4.5$
	4	$\left. \begin{matrix} 19+14 \\ 19+14 \\ 19+14 \\ 19+13 \end{matrix} \right\} 19+14$	6.5	$\left. \begin{matrix} (?)8 \\ 5 \\ (?)7 \\ 5 \end{matrix} \right\} 6$
III (c)	20.9 (air)	$\left. \begin{matrix} 14 \\ 14 \end{matrix} \right\} 14$	—	$\left. \begin{matrix} 13 \\ 3 \end{matrix} \right\} 8$
	0.75	$\left. \begin{matrix} 19+9 \\ 19+9 \\ 19+6 \\ 19+8 \end{matrix} \right\} 19+8$	5+8	$\left. \begin{matrix} 4 \\ 4 \\ 4 \\ 4 \end{matrix} \right\} 4$
IV (ii)	20.9 (air)	$\left. \begin{matrix} 19+8 \\ 19+11 \end{matrix} \right\} 19+9.5$	—	$\left. \begin{matrix} 6 \\ 6 \end{matrix} \right\} 6$
	24	$\left. \begin{matrix} 19+13 \\ 19+7 \end{matrix} \right\} 19+10$	0	$\left. \begin{matrix} 6 \\ 6 \end{matrix} \right\} 6$
	55	$\left. \begin{matrix} 19+7 \\ 19+13 \end{matrix} \right\} 19+10$	0	$\left. \begin{matrix} 7 \\ 6 \end{matrix} \right\} 6.5$

At each reduced oxygen concentration an increase is seen in the length of time at 68° F. before ripening starts. In the oxygen concentrations higher than that in air no acceleration in time of incidence of ripening was obtained. No consistent difference in the 'eating ripe' period (from time of full yellow skin colour to appearance of anthracnose pustules) at 68° F. was produced

by the different oxygen atmospheres. Whatever the oxygen concentration used at 53° F., the onset of colour change from green to yellow coincided with the rise in respiration rate, full yellow skin being attained after the peak value.

A comparison of Figs. 2, 6, 9, and 12 gives data on the effect of chilling on the trend of respiration. Figs. 6 and 12 show the 'normal' respiration and internal gas concentration curves for bananas not subjected to chilling, whilst Figs. 2 and 9 give flattened respiration-rate curves consequent upon the initiation of ripening at 53° F., with transfer to 68° F. apparently during the climacteric rise. Very flattened respiration-rate curves, in bananas of similar grade due to the climacteric being passed at 53° F. continuously, have been given elsewhere (Leonard and Wardlaw, 1941, Figs. 16, 17, 18, and 19). Complete data are not yet available on other aspects of chilling, e.g. the effect of different humidities at 53° F. and of temperatures below 53° F.

VI. DISCUSSION

Consideration is given here only to previous investigations on the effect on bananas of different concentrations of oxygen *in the absence of carbon dioxide*. In every instance (except Geerlings, 1908, and Wardlaw, 1940) the experimental material has been bananas after an overseas shipment of a fortnight or 3 weeks at low temperature. The initial physiological condition of the fruit is therefore different from that used in Trinidad.

As early as 1903 Gerber had noted that an increased percentage of oxygen increased the respiratory quotient and hastened the maturation of unripe bananas, but decreased the respiratory quotient of ripe bananas.

In Java, Geerlings (1908) found that bananas (? at tropical temperatures, 82.4° F. (28° C.)) in a tube through which nitrogen was passed remained green and hard during the period of ripening of other bananas in the ordinary atmosphere.

Using Gros Mjchel bananas losing their green colour after 3 weeks' shipment, Kidd and West (1932) found that, at 64.4° F. (18° C.), decrease in concentration of oxygen in the external atmosphere from atmospheric down to 5 per cent. had very little effect upon the rate of carbon dioxide production, but the change in colour was retarded. In 2.5 and 0.1 per cent. oxygen the rate of production of carbon dioxide for the first few days was not noticeably different from that in higher percentages. Subsequently there was a progressive divergence, the rate in 2.5 and 0.1 per cent. oxygen falling below that of the others. Normal ripening occurred in 5 and higher percentages of oxygen but not in 2.5 or 0.1 per cent. They state that the artificial atmospheres were applied in all probability at about the peak of the climacteric rise in respiratory activity. For this reason, as well as the higher temperature used and the previous storage history of the bananas, these records are not comparable with those recorded here and it is probable that their results were due to the opposing tendencies of the rising respiration of the ripening fruit and the depression of respiration by decreased oxygen concentration. In their

experiments green Cavendish bananas, at the same temperature and 80 per cent. R.H. and after similar overseas shipment, showed a retardation of ripening in oxygen concentrations below 15 per cent. and an acceleration in 30 and 50 per cent. Abnormal ripening occurred in 1 per cent. oxygen. These results were confirmed later (1933). Nelson (1939), commenting on these results, says that, if it be true that bananas ripen as well in an atmosphere containing only 2.5 to 5 per cent. oxygen, 'the respiratory processes can play no essential part in ripening and one is forced to the conclusion that ethylene is connected with hydrolytic processes of ripening'. Conversely, Lynch (1935) concludes that 'the ripening process is primarily a respiratory phenomenon, the intensification of which provides energy for an acceleration of metabolism, which in turn, cuts down the length of the pre-ripening, semi-dormant phase of the fruit'. He gives 'comparative' data of respiration rates of bananas in air and 80 per cent. oxygen and states that this increase in concentration was found to 'accelerate the ripening and respiration' of the fruit which coloured nicely. While the internal flesh softened quickly, it was found to be of poor flavour as compared with ethylene-treated fruits. He suggests that a more appropriate oxygen concentration might be used with better result and states that 'pre-ripe' bananas will not ripen in the absence of oxygen.

Gane (1934) states that the magnitude of the changes in the rate of respiration of bananas (after overseas shipment) transferred from air to nitrogen depends upon the stage at which the transfer is made. At 54° F. (12.5° C.) in green bananas prior to the climacteric rise, the rate in nitrogen slowly decreased and by the 23rd day was almost zero. When the bananas were returned to air, the rate showed no tendency to increase and it was unaffected by ethylene. At any period on or after the climacteric rise the respiration fell rapidly in nitrogen, and the degree of recovery in air depended on the time the fruit was kept in nitrogen. He confirmed this later (1936) at 59° F. (15° C.). In unripe bananas on transfer from air to nitrogen at this temperature the rate of carbon dioxide production remained steady for 2 days, and then decreased slowly to almost zero after 24 days. From his Fig. 4, p. 391 (1936), this fall in respiration rate did not occur until after 7 days in nitrogen. Even after it was returned to air the fruit remained hard and green, and its carbon dioxide production did not increase. Corresponding bananas in air showed a climacteric rise in respiration rate after 3 days. He holds the view that 'in the banana the ratio of carbon dioxide production in air and in nitrogen is not far from unity: that a process of slow metabolic adjustment takes place in the absence of oxygen leading to a new equilibrium in which the carbon dioxide production in nitrogen is less than in air (0.6–0.8). Damaging effects then supervene causing a falling off in activity and loss of power of recovery on return to air.' As in the experiments of Kidd and West, the previous history of the bananas and the storage temperature used do not allow of a closer comparison with the results recorded here. At the lower temperature (53° F.) of these experiments a lower external concentration of oxygen may be sufficient for the metabolic processes resulting in liberation of carbon dioxide, without

producing subsequent abnormal ripening. The linear relation between external and internal oxygen (Fig. 15) supports this.

Gane (1935) found that the level of respiratory activity of green bananas in air at 59° F. (15° C.) was raised by ventilating with oxygen, but the amount of combustible gaseous substance produced was not changed.

Scurti and Zavanaju (1936) found that unripe bananas at 64.4° F. (18° C.) gave a respiratory quotient less than unity in 99 per cent. oxygen and greater than unity in air. It is not clear whether the data in their tables are for comparable fruit, but if this is so, considerably lower respiration rates were obtained in the first few days in oxygen than in air (7 mg./kg./hr. during first 7 days in oxygen compared with 12 mg./kg./hr. during 2nd, 3rd, and 4th days in air) and an extension of the unripe condition occurred. (Ripening commenced on the 5th day in air and 8th day in oxygen.) In 99.9 per cent. nitrogen, at the same temperature, the fruit remained green with respiration rate 6 mg./kg./hr. during the 15 days of the experiment, but normal ripening occurred on removal to air. During 15 days at 64.4° F. and in 2.5 or 5 per cent. oxygen bananas remained unripe with respiration rate 3 mg./kg./hr. and respiratory quotient greater than 1. On removal to air normal ripening occurred in 10 days and 8 days respectively. In 10 per cent. oxygen the respiratory rate was 4 mg./kg./hr. and respiratory quotient greater than 1, no ripening occurring in 14 days, but subsequently normal ripening in 8 days in air. Their data do not permit the establishment of a simple relationship between oxygen concentration and respiration rate.

In experiments on the use of ethylene on ripening of fruits Zavanaju (1936) found that at temperatures ranging from 52.7° to 54.5° F. (11.5° to 12.5° C.) and relative humidity 90–5 per cent., bananas in air and 50 per cent. oxygen ripened to yellow colour in 14 days and 6 days respectively. After 20 days all the bananas appeared equally yellow and had lost in air 7.0 per cent. and in 50 per cent. oxygen, 6.4 per cent. by weight.

Thornton (1942) found that green Gros Michel bananas, after transport to New York, coloured very slowly in 0, 5, and 10 per cent. oxygen at 62.2° F. (19° C.) and high humidity, compared with those in 20 per cent. oxygen, whilst ripening progressed more rapidly with increased percentages of oxygen from 40 to 100 per cent. In 0 per cent. oxygen fruit was slow to ripen and was of poor colour.

In preliminary experiments in Trinidad using freshly harvested 'heavy $\frac{3}{4}$ -full' Gros Michel bananas divided into clusters (subdivided 'hands') Wardlaw (1940) obtained a retardation of ripening using supplied gas mixtures of 6.16 per cent. and 12.52 per cent. oxygen at 53° F. and 100 per cent. R.H. with CO₂ maintained at approximately 0.55 per cent. 12.5 per cent. oxygen did not afford adequate retardation during the 14 days' storage and some chilling occurred, but no injurious effects resulted at 6 per cent. oxygen. He found that the less mature the fruit (as on proceeding from the large proximal to the smaller distal hand of a bunch) the greater the extent of ripening retardation resulting from storage in a supplied gas mixture; or conversely, the more

mature the fruit is on being placed in refrigerated gas storage the less control can be obtained. Control fruit in air at 53° F. showed the slow, semi-inhibited ripening on transfer to 68° F. characteristic of chilled fruit, whereas the gas-stored fruit underwent ripening at approximately the normal rate. He notes that severely chilled fruit does not show brown mottling and slightly chilled fruit only slight mottling during ripening, thus affording an additional criterion of normality in ripening. Slight chilling has been found (Section III (a) above) to give early brown mottling. As a result of experiments in which CO₂ was allowed to accumulate in the closed container he suggests that the rate of liberation of CO₂ at 53° F. is only slightly affected over a comparatively wide range of CO₂ and O₂ concentrations. On the other hand, with a decreasing concentration of O₂ the rate of utilization also decreases.

To summarize the findings of other writers it appears that any reduction of oxygen concentration below that found in air extends the period of unripeness as compared with bananas in air, whatever the temperature. On transfer to air normal ripening subsequently occurs, provided the period in low oxygen concentration has not been unduly prolonged. Abnormal ripening, if any, occurs in bananas continuously in nitrogen. Concentrations of oxygen greater than that in air produce an earlier incidence of ripening. The data on respiration rate and respiratory quotient are not in such good agreement, probably due to differences in the earlier storage record of the bananas and to consequent differences in the proximity to the climacteric. During this, very rapid increase in the rate of liberation of carbon dioxide occurs accompanied, as shown in earlier contributions in this series, by marked reductions in the internal oxygen concentration, indicating increased oxygen requirement. This diminution in internal oxygen concentration in bananas in air has been found at all the temperatures used between 53° F. and 85° F. The absence of a stimulating effect on ripening of increased oxygen concentrations, Section IV (ii) above, may be ascribed to the absence, in the experimental bananas, of the necessary predisposition towards ripening, discussed previously (Wardlaw and Leonard, 1940) in connexion with stimulation of ripening by ethylene. This is evidenced by the considerable period elapsing between the transfer to 68° F. and the incidence of the climacteric in the bananas used. Prolongation of the period in increased oxygen concentration is required to provide further data.

As stated in the introduction, the primary purpose of these experiments was to obtain data bearing on refrigerated 'gas-storage'. Differences may be found when humidities below saturation are used in conjunction with control of oxygen and carbon dioxide concentration. Long-term storage experiments using different oxygen concentrations will be required in the further elucidation of the metabolic changes involved.

In an earlier paper in this series (Leonard and Wardlaw, 1941) it was shown that whereas reduction in temperature (from 85° F. to 53° F.) reduces the internal gaseous concentration of carbon dioxide, the content of this gas in both pulp and skin remains unaltered. It is noted here that the internal

concentration of this gas is relatively constant at 53° F. in different external oxygen concentrations, and so, presumably, is the tissue content. Barnell (1943) has shown that the rates of hydrolysis of hemicellulose and starch at 53° F. are slow prior to the onset of ripening changes but that, once these have commenced, the new, increased rate of hydrolysis of hemicellulose is apparently unaffected by temperature, whereas that of starch is. This differential effect of temperature results in a high starch:hemicellulose ratio in the pulps of chilled bananas. No data are yet available on the effect of different oxygen concentrations on the rate of hydrolysis of hemicellulose in bananas, but it is possible that low concentrations may reduce the rate. This would tend to restore the starch : hemicellulose ratio obtaining in 'normally' ripened fruit.

Subsequent studies in this series should be concerned with the effect of different concentrations of carbon dioxide upon the behaviour of bananas at 53° F. and on subsequent transfer to air at 68° F. These would yield further data on the practical problem of refrigerated storage in controlled atmospheres ('gas-storage').

VII. SUMMARY

The respiration of 'heavy $\frac{3}{4}$ -full' Gros Michel banana fingers at 53° F. (11.67° C.) has been investigated during 19 days from harvesting in an atmosphere saturated with water-vapour and containing various concentrations of oxygen, with carbon dioxide absent, such conditions being an approach to storage in controlled atmospheres ('gas-storage'). In each instance the respiration in air (20.9 per cent. oxygen) of control fruit from the same hand has been determined.

The respiration of the fingers was subsequently followed in air at 68° F. (20° C.) to determine the effect on ripening of the previous storage environment.

In decreasing concentrations of oxygen below that in air the rate of liberation of carbon dioxide is reduced, the rate in nitrogen containing less than 1 per cent. oxygen being about 6 mg./kg./hr. compared with 14 mg./kg./hr. in air. At oxygen concentrations greater than that in air a slight increase in the rate of liberation of carbon dioxide was found.

The internal concentration of carbon dioxide shows little difference in the various external oxygen concentrations at 53° F. The internal oxygen concentration shows a linear relationship with the external oxygen concentration.

For each reduced oxygen concentration at 53° F. an increase was found in the subsequent period at 68° F. before ripening changes began, compared with the control in air throughout. No acceleration of the time of incidence of ripening in oxygen concentrations greater than air was found. Normal ripening changes appeared to take place at 68° F. after the period at 53° F., whatever the previous external oxygen environment.

Comparison of the respiration of control bananas in air at 53° F. and subsequently at 68° F. provides data on the phenomenon of 'chilling'. No correlation has been found between the respiration rate, the time of incidence of ripening, and the initial weight or pulp/skin weight ratio.

The implications of these data are discussed in relation to the findings of other writers, mainly in temperate countries and using bananas after overseas shipment.

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Infection of Species of *Melandrium* by *Ustilago violacea* (Pers.) Fuckel and the Transmission of the Resultant Disease¹

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With three Figures in the Text

INTRODUCTION

THE smut fungus which attacks members of the Caryophyllaceae has been referred to both as *Ustilago violacea* (Pers.) Fuckel and *U. antherarum* Fr., the former being the more usual. Liro (1924) has broken up this species and has given the name *Ustilago lychnidis-dioicae* (De C.) Liro to the strains attacking *Melandrium dioicum* (L. emend.) Coss. and Germ. and *M. album* (Mill.) Garcke.

The mycelium of the fungus ramifies throughout the tissues of the host plant, producing brand-spores only in the anthers. Where the fungus is present in a pistillate plant, its action is to cause the development of the staminal rudiments, the anthers of which become filled with brownish-purple masses of the spores. The ovaries of such plants abort more or less completely. The presence of some uninfected flowers on such plants shows that they are modified pistillate plants. Shull's statement (1910) that they were staminate plants in which the disease had modified the dominance of the 'male' character was retracted a year later (1911). In originally staminate plants the infection causes no disturbance of flower-structure, merely replacing the pollen with brand-spores.

The mycelium perennates within the host plant, so that an infected plant appears smutted each season. Liro (1924) reported that a smutted plant of *M. dioicum* showed smutted flowers for four successive years, although smutted plants of *M. album* kept by the author have shown a progressive decline in vitality after the first season. Doncaster (in Shull, 1912) believed that plants of *M. album* might grow faster than the fungus and, sometimes, show uninfected branches as a consequence.

An inspection of the recent literature in English dealing with the method of infection (especially Gwynne-Vaughan and Barnes, 1937, and Leach, 1940) gives the impression that transmission by seed is entirely responsible. Serious consideration of the problem raises considerable doubt as to the validity of this theory (which originated with Brefeld), and investigation of continental

¹ Most of the substance of this article has been abstracted from a thesis approved for the degree of Ph.D. of the University of London (Baker, 1945).

literature shows that, in actual fact, the position is not as simple as these authors have stated. It is surprising that such well-informed authors as Gwynne-Vaughan and Barnes should have neglected the excellent review in Liro (1924). For this reason any student turning first to the literature in English receives a totally incorrect impression of the state of existing knowledge upon the subject. As no statement of the different theories exists in available works published in this country or America these theories are summarized before presenting the original work.

EARLY LITERATURE

Brefeld (1883) conjectured that the mycelium derived from brand-spores infected young plants or the more attenuated shoots of older plants, in the first case in the soil. Roze (1890), Vuillemin (1891), and Oudemans (1893) opposed this, independently, and postulated a bud-infection. Apparently Vuillemin (l.c.) was the first to attempt to correlate the staminal fruiting with distribution by insect-visitors to the flowers. Plowright (1892) supported this.¹

Controversy between authors continued. Brefeld and Falck (1905) published an account of a seed-transmission theory. Brefeld dusted spores in pistillate flowers of *M. album* and sowed the seed derived from these flowers. He claimed that 20 per cent. of the resulting plants were smutted. This is the experiment which has been seized upon by the English-speaking authors, even though Hecke (1907) tried to repeat the results, completely without success.

Brefeld's theory is that the spores find the stigmatic secretions a favourable medium for germination and growth. The mycelium so produced penetrates the young ovary and, presumably, spore-formation is supposed to take place again so that the seeds carry spores on the outer surfaces of their coats. Neger (1913) believed that the fungus overwintered in the seed although it has never been found there. Zillig (1932) denied the existence of the seed-transmission which Brefeld believed to occur naturally.

Recently Leach (1940) has reiterated Brefeld's theory, using information which would not appear to be first-hand, for he states that 'The spores are approximately the same size as the pollen-grains'. Actually they are about six times as small in diameter as the pollen grains of *M. dioicum* and nearly eight times as small as those of *M. album*.

Hecke (1907) established the possibility of shoot- and bud-infection. He demonstrated the former by cutting two-year-old plants of *M. album* down to the root-collar, painting the cut surface with brand-spores, and sealing with smutted compost. These plants produced smutted flowers the next May. By painting cut stocks (at ground-level) Zillig (1921) obtained 30 per

¹ Previously (1889) he had made the remarkable statement: 'When this fungus attacks the anthers of *Lychnis diurna* (= *Melandrium dioicum*) a plant which is uni-sexual, the styles which would normally be short, acute and erect, become long and recurved as they are in the female flower. This has been pointed out by M. Cornu as well as by other observers.' This is a complete reversal of the facts. Ainsworth (1937) and Sampson (1940) refer to this publication without pointing out the error.

cent. infection. However effective these means may have been, they have little ecological significance, although Williams, Oyler, White, Ainsworth, and Read (1939) believe that this method might be responsible for the infection of greenhouse carnation plants when they are 'stopped'.

Brefeld (1912) held that the penetration of mature tissues from spores on the surface was unlikely and, in this connexion, it is noteworthy that Doncaster (in Shull, 1912) had difficulty in infecting mature plants of *M. album*, even after scraping off the epidermis.

Werth (1909, 1910, and 1911) confirmed the possibility of seedling-infection. He demonstrated the infection of young shoots in addition. This shows itself after six to eight weeks (vide Zillig, 1932). Werth (1911) showed the existence of true flower-infection. Brand-spores were dusted in both pistillate and staminate flowers and the resulting mycelium penetrated the tissues and caused infection of neighbouring (and later) flowers. Zillig (1921) repeated this operation and obtained smutted flowers four weeks later upon the same shoot. The infections spread gradually throughout the plant. Finally, Hecke (1926) described his experiments upon the inoculation of leaf-axil buds. Successful results were obtained in every case by brushing fresh spores upon previously moistened buds.

The confusion that surrounds the method of infection is apparent. It is necessary to sort out not only the possible methods but those whose natural occurrence is probable. With this end in view experiments were undertaken and field-observations made. These disclosed that there are several points of general description of infected plants which have not been published previously.

MORPHOLOGY OF INFECTED PLANTS

Height

The most efficient parasite disturbs the life of its host in the least possible manner and, at first glance, it would appear that *Ustilago violacea* deserves this reputation. However, field evidence has shown that plants which are systematically infected are much shorter than uninfected plants or those which are known to be only recently infected.

In the experimental plot at Sunbury two rows of plants of *M. album* were obtained in 1942 from seed sown in the autumn of 1941 and the spring of 1942. Originally these were uninfected, but many plants became infected during the flowering season. In 1943 the plants formed flowering shoots again, but the shoots of the infected plants were less strong and did not reach the same height as those of the uninfected ones (15 to 43 cm. against 50 to 55 cm.). It is probable that infected plants are less fitted for natural competition than are uninfected ones.

Anthocyanin content

Generally speaking, infection of a plant reduces the amount of anthocyanin in the vegetative parts compared with uninfected plants in a similar situation. This phenomenon may be observed also in partially infected plants where

shoots bearing smutted flowers show less anthocyanin than those bearing normal flowers and show paler green leaves. No difference in the hairiness of infected and uninfected plants was observed, so that the paler colour of the leaves is not due to increased hairiness. The calyces of smutted flowers, also, are much less red than those of normal flowers. In *M. dioicum* and the hybrid there is some evidence that the infection reduces the intensity of the reddish-purple coloration of the petals.

Branching

In pistillate plants infection produces a copious branching and diminution in the size of the leaves in the region of the inflorescence. The flowers which are produced upon these thin branches are grouped into more or less sessile clusters. These are 'secondary sexual characters' of staminate plants, and their expression in pistillate plants is another example of the 'sex-reversing' action of the fungus, most obviously displayed in the development of the stamens and abortion of the ovary. The local nature of this effect should be emphasized, parts of a pistillate plant which are free from infection showing normal, stouter stems, larger leaves, less marked branching, and longer pedicels.

Flower opening

There is evidence that infection causes the premature opening of flowers, and it has been noticed that the first flowers of *M. album* to open in any season are smutted. Inspection of smutted staminate flowers of both species and of the hybrid shows that the calyx is generally split down one side, demonstrating that the flower has opened before the calyx has expanded sufficiently. This phenomenon is not seen so frequently with smutted pistillate flowers, for here the calyx, retaining its characteristically balloon-shaped form, is usually sufficiently voluminous to withstand the pressure. Infection appears to cause slight dwarfing of the calyx in both species and in both pistillate and staminate plants.

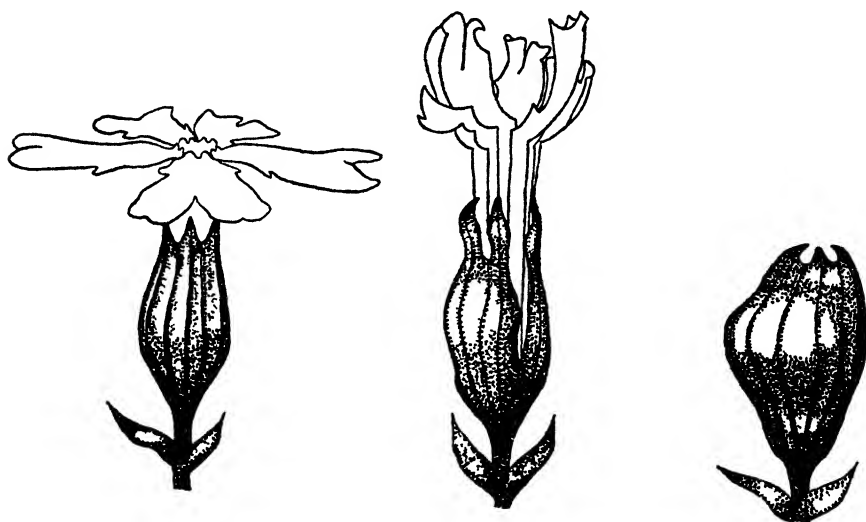
Another symptom of infection and, probably, the one responsible for the splitting of the calyx, is the great elongation of the claws of the petals. This is linked with a failure of the laminae of the petals to expand as fully as in non-smutted flowers. Thus, smutted flowers have a smaller diameter (Figs. 1 and 2). As the amount of anthoxanthin formed in each flower of *M. album* appears to be independent of the area of the lamina, infected flowers are creamier in colour than normal. An appreciable internode may be found between the calyx and corolla of smutted flowers. This is a staminate character.

^ Smutted flowers may be detected, before they have opened, by their swollen, often asymmetric shape. Such a flower-bud is shown in Fig. 3. Although the calyx has not opened, the filaments of the stamens and the claws of the petals have lengthened already, and have caused the bulge to one side. In a normal flower both the elongation of the filaments and the dehiscence of

the anthers take place in a regular order. In a smutted flower all are dehiscent and of about the same length before the flower opens. Furthermore, the anthers of smutted flowers are more swollen before dehiscence than normal. These observations apply equally to both species.

Scent

Smutted flowers of *M. album* still emit their scent in the evening and, consequently, they are still visited by moths. Probably this is important for the transmission of the brand-spores to new hosts.



FIGS. 1-3. *Melandrium album*. Fig. 1. Normal staminate flower. Fig. 2. Smutted pistillate flower, showing elongated petal claws, incompletely expanded petal-laminae, and split calyx. Fig. 3. Bud of smutted staminate flower.

EXPERIMENTAL TESTS OF THEORIES

Plants of *M. dioicum*, *M. album*, and the F_1 hybrid between them were transferred to pots in the greenhouse during the early summer of 1942. Smut spores were obtained from the flowers of infected plants of *M. album* and from a plant which was determined to be a back-cross of the hybrid to *M. dioicum* (vide Baker, 1945a, p. 41). They were dusted copiously upon the stigmatic surfaces of open pistillate flowers with a camel-hair brush, care being taken to avoid infecting other parts of the flower or plant. Pollination of the flowers was performed by the same means. Careful exclusion of possible pollinating insects was maintained. All possible combinations of pollen- and smut-donors were made. The seeds from the resulting capsules were examined. They showed no evidence of any smut-spores upon the seed-coat and no spore-formation could be detected in the capsules. The seeds were sown immediately in the experimental plot and produced many plants before the end of the summer. By the summer of 1943 all had flowered and showed no

smutted flowers whatever. This is in direct contradiction to the results of Brefeld (1905) but in complete agreement with those of Hecke (1907).

Brefeld's theory demands that the brand-spores germinate to form promycelia upon the stigmatic surface. From these basidiospores would be cut off and conjugate. The binucleate mycelium formed from this would be the infecting agent and grow down the styles to the ovary where the theory requires the formation of brand-spores to occur again. These would be carried on the seed-coat and the whole process repeated in the soil before the binucleate mycelium could penetrate the seedling.

There are several objections to this theory. It seems rather surprising that fruiting in the ovary could be expected to occur without causing abortion, for the effect of the fungus upon a pistillate plant when invading the flower from the vegetative region is to cause abortion of the ovary and ovules and development of the staminal rudiments. The same effect might be expected when the hyphae arrive from the styles. The conclusion of Erlenmeyer and Geiger-Huber (1935), that the 'sex-reversing' effect of the fungus is due to a hormone which it produces in growth, supports this objection (vide also A. and D. Löve, 1945).

To see if fruiting ever takes place in the ovary, the ovaries borne by many smutted pistillate plants growing in the experimental plot were examined. These were in various stages of abortion, but in no case was any spore-formation seen within them although it was taking place copiously in the stamens of the same flowers. Normal flowers from adjacent plants were examined, also, with the same negative result. In a corresponding examination of wild plants, one plant only showed any remarkable features. This was a specimen of *M. album* growing in 1942 upon a grassy bank at Stanwell, Middlesex. It was a pistillate plant which had become smutted. The 'sex-reversing' influence of the fungus did not express itself as completely as usual and, although stamens were formed and spore-formation occurred in the anthers, the filaments were only partially elongated. The most striking feature was the development of small capsules (about 0.5 cm. in length), which became woody and opened by five pairs of teeth. Nevertheless, all of the ovules aborted and produced no seed. An uninfected portion of the same plant produced normal capsules containing clean seed. Although the ovules in the infected flowers had aborted, it was discovered that there were spores intermixed with the glandular hairs which occur within the ovary. In this case, then, there *was* fruiting of the fungus, but its accompaniment by abortion of the ovules is significant.

An apparently similar plant, collected by J. Gay from Ried, near Zermatten, is to be found in the herbarium at Kew. It is of *M. dioicum*. The claws of the petals are very long, the very small capsules are about 0.6 cm. in length and not swollen. The flowers are blackened. Gay remarks that the stamens were sometimes perfect and sometimes aborted, while the ovaries were 'perfect' although the styles were short. It is possible that some seeds *may* have been produced by this plant, for there is no evidence that the three

seeds exhibited separately might not have come from a non-smutted portion of the plant. Seed borne in the capsules produced by clean flowers on infected stock in the experimental plot was germinated and produced clean plants only. This confirms an observation by Werth (1911).

Brefeld's theory demands that infection of the seedling shall take place at a very early stage. This means that the formation of the binucleate mycelium must take place in the soil and that this mycelium, upon which the infection of the host depends (Gwynne-Vaughan and Barnes, 1937), must be present at the right time. The likelihood of this being the case must be considered.

Plowright (1889) stated that the germinative faculty of the spores lasted for 6 weeks, but Werth (1911) maintained that brand-spores kept at summer temperature were capable of germination a year later. Liro (1924) confirmed this. Also, Werth showed that after exposure to two severe frost-periods, subsequent soaking of the brand-spores in water gave numerous germinations several days later. Schopfer and Blumer (1938) demonstrated that the fungus made good growth in culture at 1° C. and noted that this might explain the capacity of spores to overwinter in the soil. Zillig (1921) found that the spores still lived after five to ten months' desiccation. The results of Werth, Zillig, and Liro have all been obtained from spores that have been stored dry. However, the conditions in nature are likely to be different. Damp periods are likely to cause germination of the brand-spores and subsequent inclement conditions would be much more likely to be lethal.

Seed of *M. album* was dusted very liberally with brand-spores from smutted flowers of the same species. The resultant concentration of spores upon the seed-coats was far in excess of anything possible naturally. In addition to this dry dusting, moistened seeds received similar treatment. These seeds were sown in the experimental plot in September 1942. They did not germinate until the spring of 1943, but the plants which they produced flowered through the summer and all were perfectly clean. On April 9, 1943, the process was repeated. Of all the plants which developed, only one (of the moist inoculation) bore smutted flowers. This infection was systemic and, undoubtedly, derived from the spores on the seed-coat. However, it was the only plant among twenty of that batch which was infected despite the exceptionally heavy inoculation. It seems that infection from spores carried upon the seed-coat is unlikely in nature even if they could be expected to be there.

The alternative theory, which has already been suggested by Werth, is that flower-infection does take place but that the mycelium eventually derived from the spores grows past the ovary and penetrates the vegetative tissues of the host. Werth (1911) and Zillig (1921) succeeded in performing this experimentally and caused the infection of neighbouring flowers. In the experimental plot at Sunbury and in nature this process has been observed repeatedly by the author in the last five years (see p. 340).

In the experiments cited above with the artificial introduction of brand-spores to flowers in the greenhouse (p. 337) the results were not what might have been expected with the experience of this natural flower-infection and

the results of Werth and Zillig. Nevertheless, it must be emphasized that care was taken to confine the spores to the stigmatic surfaces as far as possible. There is no evidence as to what precautions were taken by Werth and Zillig in their successful experiments. Werth (1911) stated that the brand-spores do not germinate upon healthy styles but do so only when the flowers have become mushy, while Liro (1924) noted that germinated spores can be observed, easily, in older flowers. Werth (1911) used his observation to show that the contention, subsequently re-made by Neger (1913), that the mycelium might penetrate the developing seed and perennate therein was most unlikely as the seeds would be too old by the time that the mycelium reached them.

In view of the negative results obtained by the author, the opinion held by the previous experimenters that the spores do not germinate upon the styles until the flower is old would seem to be correct. An infection was obtained in the experimental plot by scraping the surfaces of the styles and dusting them with spores, but the penetration of some other part of the flower is probable, naturally. Provided that the osmotic pressure of the nectar is not too great, there seems no reason why the infection could not take place through a nectary, while the gynophore or the cells of the claws of the petals are other possible sites for entry.

With this possibility in mind, spores were dusted in the bases of the flowers of two pistillate and two staminate plants of *M. album*. On each of the plants smutted flowers were produced eventually.

Apart from true flower-infection, the frequency of occurrence of the other established methods of infection must be considered. The author has noticed that leaves from beneath smutted flowers have spores lying upon them and examination of the stems of infected plants has shown adherent spores. The presence of glandular hairs helps in their retention. Thus there is quite a considerable possibility of self-infection of young shoots taking place after the smutting of a single flower or group. Werth (1911) and Zillig (1921) have postulated that spores will fall upon the ground beneath the plant and, after over-wintering, cause the infection of young, elongating leafy stolons.

In the experimental plot the author found that seedlings (from clean seed) allowed to appear beneath an infected plant showed a high percentage of infection. As they had reached above the soil-surface when the first smutted flowers appeared upon the presumed donor plant, it is most likely that bud-infection was operative.

FIELD OBSERVATIONS AS TESTS OF THE THEORIES

In the experimental plot, plants of *M. album* derived from clean seed flowered throughout the summer of 1942. To begin with, the flowers were normal in every way and pistillate plants showed no 'staminate' characters whatever. In the later part of the summer, however, *groups* of smutted flowers appeared upon shoots which, previously, had been bearing unsmutted flowers. There can be no doubt that in each of these cases brand-spores had been brought by insect-visitors to the clean flowers and that the derived mycelium

penetrated the vegetative tissues and caused the infection of the developing flowers on the same shoot. In some cases the infection spread to neighbouring shoots upon the same stem, but the infection did not become systemic in 1942. The aerial portions of the plants died down to soil-level, as usual, in the autumn, and in 1943, when flowering shoots were produced again, they bore smutted flowers. Not every shoot bore smutted flowers, however, and in a few plants where infection had taken place too late in the season the mycelium did not appear to have penetrated to the rootstock before the aerial portion died back, with the result that uninfected flowers were borne the next season.

In addition to these plants of *M. album* in which the spread of infection was observed, many plants in nature showed evidence of the same phenomenon. Many pistillate plants bore capsules containing mature seeds upon the same shoots as infected flowers with aborted ovaries, showing that seed-production had been normal before infection took place. The grouping of the flowers into almost sessile clusters with dwarfing of the stem-leaves followed the infection. The further development of anthocyanin in infected shoots was restricted and the infected flowers had characteristically paler calyces. This development of disease has been noticed, also, in two plants of calculated hybrid ancestry growing in nature at Burhill, Surrey, and Lower Halliford, Middlesex, respectively.

The spread of infection through staminate plants has been observed, in addition, beginning in one group of flowers and spreading to neighbours. There is no doubt, therefore, that infection can take place by this means and that it is of frequent natural occurrence.

A plant of *M. dioicum* was found near Weybridge in the summer of 1942. This plant bore capsules (empty at the time of examination) at the apex of one shoot, but the lateral branches had produced smutted flowers. In the 1945 and 1946 seasons the spread of infection in plants of this species has been observed, repeatedly, in Yorkshire.

During field-work it was observed that the proportional incidence of infection of *M. album* decreased from the east and south-east of England to the south-west and Wales while a similar decrease occurred northwards. With certain exceptions, which are discussed later, smutted plants of *M. album* are unknown to the author in Glamorgan and Yorkshire where observations have been made. This is correlated with a decrease in frequency of the host and would be expected, especially if true flower-infection is the prevailing method. Where the plants are not common, bud-, shoot-, and seedling-infection are out of the question. With true flower-infection, as opposed to seed-transmission, the case is that the seed which reaches these places is clean and gives rise to clean plants. Either the plant is not visited by pollinating insects, in which case it certainly remains clean and, even if the plant is pistillate, produces no seed, or else the insect-visitor comes from another relatively isolated plant which, similarly, is likely to be clean and no infection is to be expected. If transmission were by seed, however, the seed which established the isolated plant might be smutted and give rise to an infected plant. If this were visited

by nectar- or pollen-seeking insects, the smut spores would be carried away, in some cases to other relatively isolated plants and the seed of these plants would be infected. With both means of transmission, the fact that flowers are visited less frequently by insects which have visited another plant of the same species previously means that there is less chance of the smut spores being brought to them. Nevertheless, there is less reason to expect complete freedom from infection with the seed-transmission theory than with true flower-infection.

M. dioicum shows a similar condition. This species is commoner in northern and western Britain than in the south-eastern counties. In Surrey and Sussex only a few smutted flowers have been seen by the author and there is evidence that they have had a hybrid ancestry. In the north and west of this country, however, the infection of plants of *M. dioicum* of apparent purity is common. It is noteworthy that the only references in British local floras to plants of *M. dioicum* bearing smutted flowers appear in Lees (1888), Massee and Crossland (1905), Horwood (1919), and Ridge (1929). The first two of these references are to floras which deal with Yorkshire and, in both cases, infection of *M. album* is not mentioned. That this approximates to the true state of affairs has been confirmed by the present author. The third reference is an author who has had most of his experience in the Midlands. He reports the infection of both species as does the last author in the list in his flora of north Staffordshire. Surprisingly, Wheldon and Wilson (1907), in their flora of west Lancashire, mention infection of *M. album* only.

In the study of herbarium specimens from Britain, the only obviously smutted specimens of *M. dioicum* which were seen were from Jervaulx in the North Riding (J. E. Little in Herb. Univ. Cantab.) and Grassington in the West Riding of Yorkshire (W. A. Sledge in Herb. Leeds Univ.), Milltown, near Ashover, in the north-east of Derbyshire (C. Bailey in Herb. Mus. Brit. (N.H.)), and Oldbury Court Woods, Bristol, West Gloucestershire (Ida M. Roper in Herb. Leeds Univ.).

Near Llanrhidian and Rhossili, in the Gower peninsula of Glamorgan, where *M. dioicum* is very common, two populations of the species were observed with more than half the plants bearing smutted flowers. These populations showed no sign whatever of hybridization and *M. album* is known only as a rare introduction in the Gower.

The demonstration that infection is proportionately *least* common where the species concerned is least common, even though the other species may be very common in that region, indicates that two separate strains of the fungus exist. This has been maintained, already, by Zillig (1921), who considered that at least eight strains of *Ustilago violacea* exist, restricted to different members of the Caryophyllaceae. He was able to transfer the strain attacking *M. album* to *Agrostemma githago* and *Silene noctiflora*. Another strain which did not attack *M. album* attacked *M. dioicum*. This postulate has been challenged by Liro (1924), who said that spores from *M. album* and *M. dioicum* could infect either of the species. Kolk (1930-1) has questioned the reliability

of Zillig's conclusions, also, because he used less than fifty plants in nearly every case. Zillig himself stated (1921) that he had infected both *M. album* and *M. dioicum* with spores from a smutted plant of *M. album* × *M. dioicum*. This would make it appear that the hybrid can act as a 'bridging host' (but cf. Reed (1935), who has discussed the question of bridging hosts and points out that the phenomena may be explained by the selection of specialized races from an original mixture). Goldschmidt (1928) made hybridizations between races of the fungus and deduced that specialization was inherited in a Mendelian manner.

Obviously this question is of considerable ecological importance. In the experimental plot, flower-infection by the agency of pollinating insects and bud-infection were both observed with *M. album* (see above). The rows of plants which were attacked were interspersed with rows of plants of *M. dioicum*, but in no case did any of the latter show smutted flowers. This was not due to any specific choice by the pollinating insects, for they were observed to visit both species.

In the relatively few cases where infection of plants of *M. dioicum* was seen in Surrey and Sussex (Table I) (none was seen in Middlesex), inspection of the data presented elsewhere (Baker, 1945) will show that hybridization has been marked.

TABLE I

Smutted Plants of M. dioicum in South-eastern England

Localities of infected plants of <i>M. dioicum</i> .		County.
Alder Wood, New Haw	}	Surrey
Stream bank, Weybridge		
Ash Wood, Poynings	}	Sussex
'Woodland relic', Saddlescombe		
Alder Wood, Newtimber		
Danny Park, Hurstpierpoint		
Grass bank, Clayton		

In the Students' Garden at the Royal Botanic Gardens, Kew, during the 1942 season, the plants of *M. album* contained some which were infected, while the plants of *M. dioicum* did not. The patch labelled as the hybrid contained plants of generations other than the first, and some of these were smutted. As reported above, natural infection of two hybrids was observed to occur and, in another case, at Tillington, Sussex, a calculated hybrid was seen to be smutted. All of these plants were growing in the midst of plants of *M. album*.

In experiments conducted at the University of Leeds the author has found it easy to infect plants of *M. dioicum* with spores from infected plants of the same species growing at Meanwood, Leeds. Although leaf-bud infection, flower-infection, and the painting of spores on flowering stems from which the epidermis had been scraped were all tried with *M. album*, no infection was obtained. The only plants of *M. album* in Yorkshire seen to be infected in 1945-6 were growing with hybrids in a field near Collingham. Thus there

appears to be considerable evidence that specialized races *do* exist and that one effect of hybridization is to introduce susceptibility to infection by the strain usually attacking one species only into populations of the other species which were immune previously.

The general ecological importance of the attack by *Ustilago violacea* is great. The attack renders a plant more or less sterile and this must have a profound effect upon the capacity of the species for withstanding competition. Zillig (1920) found in 1919 and 1920 that about 21 per cent. (out of 3,569) of the plants of *M. album* which he examined were infected, although with *M. dioicum* extensive stands were healthy and only at fewer places was infection shown.

The incidence of the disease in different populations of *M. album* was studied by the present author during the months of (late) April, May, and June of 1944. At this time of the year only those plants which were systemically infected would be likely to show smutted flowers; later on in the season insect-transmitted infection (of that season) would show. The results obtained by counting samples are shown in Table II. The results from populations which are in proximity are grouped together.

TABLE II

Populations of M. album investigated in South-eastern England

Population.	Description.	Disturbed regularly or not.	Percentage of plants smutted.
Waterworks Bank, Sunbury	Waste bank	No	35.3
Pasture to south of Fordbridge Rd.	Pasture	No	51.9
Fordbridge Rd.	Hedge bank	No	13.2
Field No. 1, Halliford cross-roads	Arable land	Yes	0.0
Field No. 2, Halliford cross-roads	Arable land	Yes	0.0
Railway Bridge, Upper Halliford	Grass bank	No	67.3
Path to Sunbury Lock	Hedge and grass bank	No	27.8
Bank to west of Sunbury Lock	Grass bank	No	40.0
Norwood Farm	Waste ground	No	66.7
Entrance to Burhill Golf Course	Waste ground (grassy)	No	58.6
Seven Hills Road, Walton	Grass heath	No	42.9
Seven Hills Road Allotments	Allotments	Yes	0.0
Varndean School Field, Brighton	Arable land going fallow	Yes, till 1942	0.4
Varndean School Field, Brighton	Weeds on portion cultivated as allotment	Yes, including 1944	0.0
Hollingbury	Waste land and hedgerow	No	33.2
Roedean Cliffs	Grass on cliff-top	No	20.0
Brapool, Brighton	Roadside verge	No	44.4

The results demonstrate that infection is marked where the plants are undisturbed by agriculture and the infection is allowed to spread through the population. Where the population is decimated frequently, as in the arable

fields at Halliford (and here many thousands of plants were growing), not a single infected plant was to be found, although in the hedgebanks (Fordbridge Road) lining one of the fields and near to the other the infection was marked and was severe in the pasture opposite. The results from the Walton-on-Thames and Brighton areas substantiate this. The figures for percentage-infection of pistillate and staminate plants, respectively, showed no significant difference. Neither would it appear that the incidence of the disease varies materially with the subsoil.

These results support the contention that infection by seed does not occur in natural populations and that spores are transmitted between mature plants. A similar conclusion may be drawn from the rarity (if not complete absence) of the disease in North America, where the species was introduced by seed.

DISCUSSION

The main theories of transmission of the infection advanced by Brefeld, Werth, and Hecke, respectively, have been tested by practical means. Brefeld's belief in seed-transmission can be subjected to severe criticism on theoretical grounds, for it seems surprising that the binucleate mycelium (eventually derived from the germination of the brand-spore upon the stigmatic surface) which penetrates to the ovary may not continue its growth into the plant to give a systemic infection but should find it necessary to fruit in the ovary, exist in the inclement soil, and go through the whole life-cycle again so that it may eventually penetrate the tissues of the seedling. These seedling tissues will hardly be easier to penetrate than those of the tip of the flower-stalk. The fact that fruiting of the fungus in the flower is accompanied, *invariably*, by abortion of the ovules, the overwhelming number of observations of the spread of infection from one flower throughout a plant following natural or artificial infection, and the failure of seeds heavily coated with spores to produce infected seedlings, sound the death-knell of this theory. The field-observations in regions where the two species are either rare or common confirm this conclusion and, together with the same theoretical and observational evidence which weigh so heavily against seed-transmission, establish the importance of true flower-infection in nature.

Self-infection through spores falling from infected flowers on to young shoots may be expected to occur. A similar deposition of spores upon young plants arising beneath an older one or the infection of leafy stolons growing up through soil containing spores will be more prevalent in populations of the gregarious *Melandrium dioicum* than the much less gregarious *M. album*. In both species, however, these processes may be expected to play a minor role in consolidating the infection in a population after it has been introduced from elsewhere by insects.

The existence of two separate strains infecting the two champions seems likely. This is of considerable ecological importance, for it has been shown (Baker, 1945, 1945a) that *M. album* is invading the regions once occupied by *M. dioicum*, alone, and is producing hybrid swarms. In this way, susceptibility

to infection by *Ustilago violacea* is being introduced into populations of *M. dioicum* where, associated with their relative rarity, the plants of this campian are uninfected. By such sterilization their precarious tenure of the land will become weakened further. In the west and north of Great Britain *M. dioicum* is frequently infected with its own race of the fungus, but here it is still a common species and the effect of the fungus is not likely to be so serious.

Many members of the Caryophyllaceae are susceptible to attack by various races and the family must suit the physiological needs of the fungus. Leach (1940) claims that 'This disease is a striking illustration of the adaptation of a fungus to insect-transmission. The association is probably a very old one and may have evolved along with the host and its adaptation for insect-pollination.' The suggestion in the latter half of the second sentence is hardly supported by the facts. Undoubtedly, the adoption of insect-pollination by the ancestors of the Caryophyllaceae preceded the association with the fungus. Furthermore, spore-formation by members of the Ustilaginales takes place in the flowers of many *anemophilous* species of angiosperms.

SUMMARY

A critical account is given of the arguments over the method of infection of species of *Melandrium* and the transmission of the ecologically important disease caused by *Ustilago violacea* (Pers.) Fuckel. These are neglected in the literature in English, and in two important reference works the accounts given are based solely on the results of Brefeld. New theoretical considerations and the results of laboratory and garden experiments together with field data are described which prove that Brefeld's seed-transmission theory is incorrect and that true flower-infection is most frequent in nature. In this, pollinating insects convey the spores from one host to another, infection of the new host taking place later through some portion of the flower. The infection of seedlings and axillary buds also may occur.

Further effects of the fungus upon the host are described and explanations are offered.

Infection in both *M. dioicum* and *M. album* appears to be most severe where the species is most common. This is related to the means of transmission of the fungus and the probable existence of two strains, one of which infects each species. The effect of interspecific hybridization in this connexion is outlined.

With *M. album*, the infection is most marked in undisturbed localities and least marked in cultivated fields.

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On the Structure and Reproduction of *Uronema terrestre* n.sp.

BY

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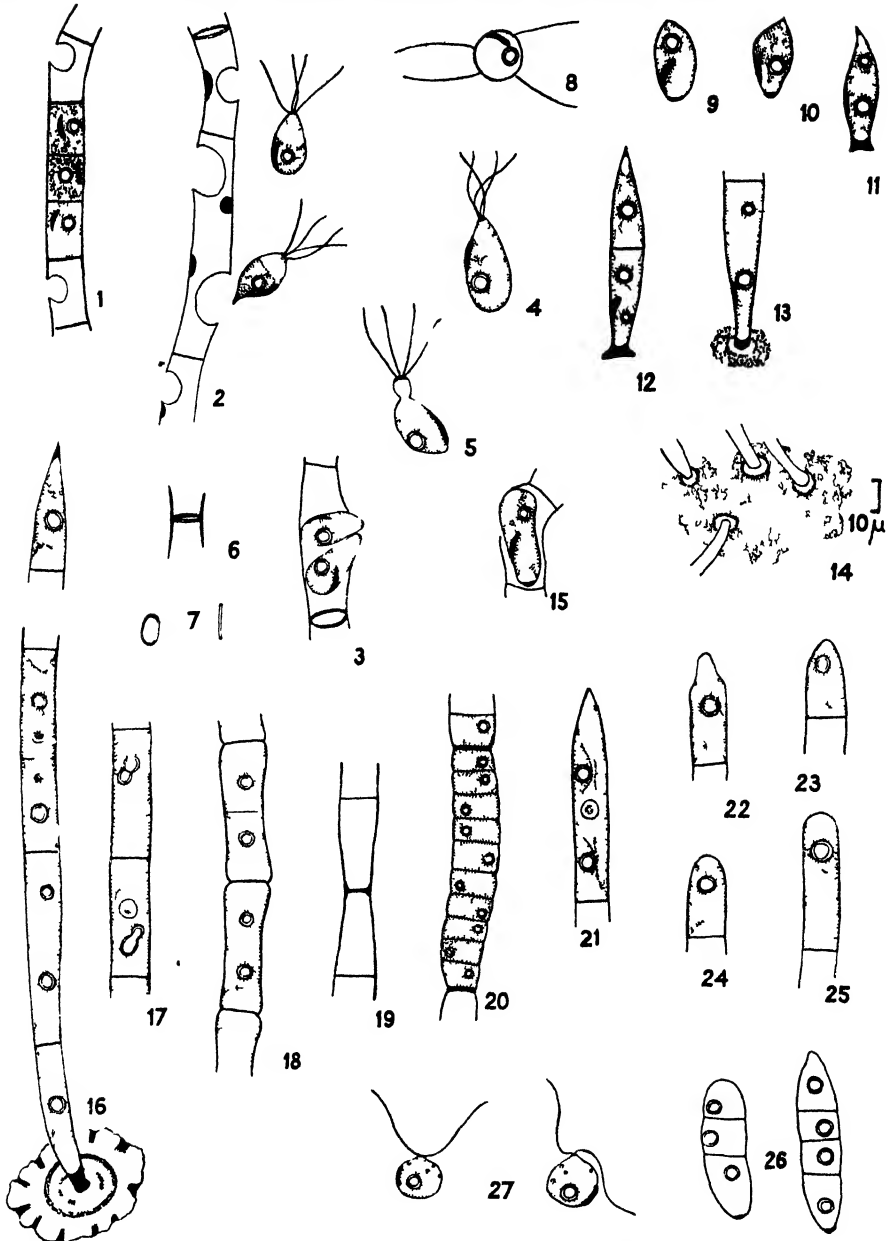
With fifty-nine Figures in the Text

THE species of *Uronema* dealt with in this paper appeared in enrichment cultures of soils from rice-fields near Allahabad, India. Cultures derived from a single thread were grown both in Benecke's solution together with a little soil extract and in De's (1939, p. 124) modification of it. It was also grown on solid media prepared by adding 1·5 per cent. agar to these solutions. It grew well in all the cultures, although in liquid media with ammonium nitrate older cultures soon assumed an unhealthy yellowish appearance and showed plentiful degenerating cells. Bacteria-free cultures were not obtained, but by repeated subculturing the bacterial numbers were kept low enough not to interfere with the normal growth of the alga.

Uronema is characterized by possessing an unbranched filament of elongate cells, the terminal one with an acuminate tip, while the narrowed basal cell is expanded into an attaching disc. Six species, including a marine form, have so far been described, but there remains considerable uncertainty regarding the systematic position of the genus and doubts have even been voiced as to its independence (Fritsch, 1935, p. 201; Gaidukov, 1903, p. 524). No detailed study of the range of variation of any one species such as is attempted in this paper has so far been undertaken.

VEGETATIVE FEATURES

The filaments of the Allahabad species, which may be called *U. terrestre*, are only 3–5 μ in diameter and of indefinite length. In liquid cultures they can reach a length of about 6 cm. and on agar they may be more than 30 cm. long. The greatest length so far recorded for any species of *Uronema* is 5 cm. (Vischer, 1933, p. 77). The terminal cell gradually narrows to a solid tip (Fig. 16) which is usually straight, rarely curved. The nearly circular attaching disc, about 20 μ in diameter, is thin and flat and has a lobed margin. In mature plants in liquid cultures it shows several concentric dark brown rings separated by light brown to almost colourless zones. In young plants the colourless central portion of the disc is surrounded by a brownish zone, while the apparently mucilaginous part beyond contains numerous colourless or brownish granules (Fig. 13). The mucilaginous portions of the individual holdfasts of adjacent germlings may coalesce to



FIGS 1-27 *Uronema terrestre* n. sp. Fig 1 Formation of zoospores. Figs 2, 3 Liberation of zoospores. Fig 4 Zoospores. Fig 5 Mutilated zoospore. Fig 6 H-piece. Fig 7 Persisting septa left after liberation of zoospores. Fig 8 Zoospore recently settled. Figs 9-13 Stages in germination of zoospores. Fig 14 Germlings with a common holdfast. Fig 15 Germination of zoospore *in situ*. Fig 16 Germling with lobed and zoned holdfast. Fig 17 Division of pyrenoid. Figs 18 and 19 Portions of filaments. Fig 20 Part of a filament showing vigorous zoospore production. Figs 21-5 Tips of filaments from agar. Fig 26 Germlings from agar. Fig 27 Biflagellate swimmers. Magnification of Fig. 14 as shown, that of other figures is the same as shown for Figs. 28-43 and 44-59.

form a uniform expanse, the hyaline zone around the base of each germling being encircled by a distinct brownish ring (Fig. 14). In agar cultures the discs are smaller and hyaline and zonation is practically absent. The holdfast is stained brown by iodine but gives no cellulose reaction with chlor-zinc-iodide such as is shown by the cell-wall. It is at best only faintly stained by methylene blue and ruthenium red, although gentian violet colours it more strongly than the cell-wall. Earlier workers have concluded that the holdfast of *Uronema* consists of pectic substances (Hodgetts, 1918, p. 160; Vischer, 1933, p. 76).

The basal cell of the filament gradually narrows towards the holdfast and it is here filled with a hyaline shining substance continuous with the central area of the disc. In other respects the basal cell resembles the ordinary cells, always containing a well-developed chloroplast and playing a role in reproduction. The terminal cell is commonly shorter than the other cells which in the normal vegetative condition are 22–35 μ long, though sometimes up to 60 μ long. In the reproductive phase they are frequently only slightly longer than broad. The older cells are wider near the septa where they are constricted (Fig. 18). They narrow down in the middle where new septa are formed. The daughter cells are, therefore, of uniform width or widened at one end only. The filament as a whole is constricted at intervals and at these points the septa are thick (Fig. 19).

The parietal chloroplast has the general form of a hollow cylinder the ends of which are so thin that a dome-shaped area of the central vacuole is seen on either side of the septum. These areas, which like the rest of the vacuole stain with neutral red, usually contain one or two rounded bodies showing Brownian movement. The main portion of the chloroplast consists of two thicker circular bands separated in the middle region of the cell by a much thinner area which appears as an oval or rounded foramen from the surface and through which the central vacuole is again recognizable. Within this central area the nucleus is often visible without staining. Each of the thicker bands of the chloroplast contains a large pyrenoid with a conspicuous starch-sheath; there is also a little stroma-starch. Very often, especially after cell-division or under conditions of weak illumination and inadequate nutrition, one or both the bands may be incomplete, although the central and terminal hyaline areas are still visible (Figs. 17 and 21).

In cell-division an ingrowing septum cuts across the thin central region of the chloroplast and gives rise to two dome-shaped areas. The daughter nucleus migrates towards the hollow of the dome and, as the cell elongates, the thin peripheral portion of the chloroplast becomes extended. On the side of the nucleus away from the septum the chloroplast thickens to form a new band which is at first incomplete owing to irregular growth of its edges (Fig. 17). The formation of the complete band takes considerable time and, when cell-division is rapid, either or both the bands remain incomplete. Sooner or later the pyrenoid becomes constricted and divides, the division often being unequal so that one pyrenoid is smaller than the other. The former becomes

lodged in the new band of the chloroplast and grows to the full size. Division of the pyrenoid is often postponed until the cell has elongated so that there may for some time be a single pyrenoid (Figs. 22-5). Rather rarely one or both pyrenoids may divide before nuclear division so that the cell contains three or four pyrenoids. Ultimately each mature cell comes to contain two pyrenoids, although in rare instances they are very small or even indistinguishable.

REPRODUCTION

In the rapidly dividing cells, formed during zoospore-production, there is very little elongation. After one or two divisions have occurred, the chloroplast loses its characteristic form and appears to fill the cell, although traces of the terminal vacuolar regions can be discerned. Such short cells contain but one pyrenoid and give rise to a single zoospore. Cells, which have not recently divided and contain two pyrenoids, invariably form two zoospores each with one pyrenoid. An elongate massive eyespot is differentiated at an early stage before the contents become organized as zoospores (Fig. 1). The ordinary cells often show two such eyespots, one near each chloroplast band. In optical section the eyespot has a convex outer and a concave or flat inner surface; the outer part is bright red, while the inner appears paler.

Liberation of zoospores takes place through a lateral aperture formed by solution of the wall at a point where a slight bulge develops. If two zoospores are formed, they may escape through the same or through separate apertures (Figs. 2, 3). The aperture is at first narrow, but after the flagella and the anterior end of the escaping swarmer have become protruded, the zoospore rotates vigorously on its axis and the aperture becomes much enlarged. The zoospore may be mutilated in various ways during escape. Most commonly the hyaline tip becomes separated by a constriction from the rest and this persists in the liberated swarmer (Fig. 5), indicating that the periplast is firm enough to allow of no subsequent recovery. In the smaller cells the entire lateral wall becomes gelatinized and the zoospores merely slip out after some movement. In some instances small thickenings appear on the wall of the empty cells (Fig. 2) which are not evident before discharge and are not found in filaments grown on agar. They do not stain with ruthenium red.

The groups of short cells produced in preparation for vigorous zoospore-formation are separated by thicker septa (Fig. 20). These, together with parts of the lateral walls, form H-shaped pieces, usually having limbs of unequal length curved outwards, after liberation of the zoospores and consequent disintegration of the filament. The remaining septa are seen as detached circular plates (Figs. 6, 7). Even after soaking in caustic potash and subsequent treatment with ruthenium red there is no indication of an H-shaped structure in the wall of the vegetative cell, and the H-shaped pieces previously described are merely due to the persistence of the thick septa and the adjoining portions of the side-walls.

The zoospores are oval or elongate-oval and possess a cup-shaped chloro-

plast with the pyrenoid placed laterally in the posterior half. They measure $6-15\mu$ in length and $5-8\mu$ in breadth. The eyespot, which projects slightly from the surface, is situated near the middle, although often displaced towards the posterior end. It is always in contact with the chloroplast and, if the latter extends into the anterior end, may be altogether anterior (Fig. 4). Two contractile vacuoles, pulsating alternately, are situated near the anterior end, where the plasma membrane is slightly thickened and from which the four equal flagella, as long as or slightly longer than the body, arise. The posterior end is commonly extended into a hyaline pointed process of varying length (Fig. 2).

All the zoospores, both large and small, are strongly positively phototactic. In the light they soon come to rest, whereas in darkness they continue to move for a long time. If particles of sand or cotton fibres are present most of the zoospores settle upon them, and there is an evident epiphytic tendency. They become attached by the anterior end, meanwhile rotating vigorously on their axes. There is only slight contraction and the recently settled zoospore is often elongate. The anterior end becomes flattened and a wall is secreted, while if the posterior end is not already pointed, it becomes acuminate. The eyespot can still be distinguished in two- or three-celled germ-lings (Figs. 11-13), but it gradually loses its colour and ultimately becomes colourless. It is mostly the posterior portion of the zoospore that elongates, since the eyespot remains at its original distance from the tip. As the chloroplast enlarges the vacuole becomes evident at the tip and at the base, the basal vacuolar area being recognizable from the earliest stages (Figs. 8, 10). The young germ-lings are mostly straight.

The formation and liberation of zoospores do not appear to follow any particular sequence, such as is reported for *U. confervicolum* (Lagerheim, 1887, p. 519) and *U. elongatum* (Hodgetts, 1918, p. 163). Any cell, even the terminal and basal cells, may produce zoospores, but usually a number of adjacent cells do so, while others in the same filament remain vegetative. A slight change in the medium, especially dilution, will initiate zoospore-formation both in mature filaments and in three- or four-celled germ-lings. The frequent record of few-celled filaments in *Uronema* in nature is, perhaps, a result of this tendency to form zoospores with a slight change of environment. Water-movements or displacement of the substratum by wind might be sufficient to cause zoospore-formation before the filament has grown to any length. According to Mitra (1945, p. 115) the filaments of *U. indicum* are about 6 mm. long in nature, while in cultures they attain a length of 4.2 cm. Vischer (1933, p. 74) records those of *U. gigas* as a few millimetres long in nature, but many centimetres long in culture. Length of filament should, therefore, not be stressed in delimiting species of the genus. *U. confervicolum*, which is not known to exceed 1 mm. in length, may in cultures be found to be capable of producing filaments of indefinite length.

Uronema is described (Printz, 1929, p. 158) as attached throughout life. In *U. terrestre* zoospore-production leads to breaking up of the filaments and

in liquid cultures most of them are free-floating and continue to grow. In nature this condition is probably rarely realized owing to early production of zoospores.

Zoospores are produced in media with a pH range of 11.5 to 3.4, although those of pH 6.0 and below are progressively less favourable. At pH 2.0 no zoospores are produced and most of the cells appear moribund. At pH 9.4 and 3.8 the germlings are straggling with disintegrated chloroplasts and small or unrecognizable pyrenoids. In *U. gigas* Vischer (1933, p. 76) found the optimum for zoospore-production to lie near pH 5.4, while at pH 7.2 and above none were formed. There is thus a physiological difference between this species and *U. terrestre*.

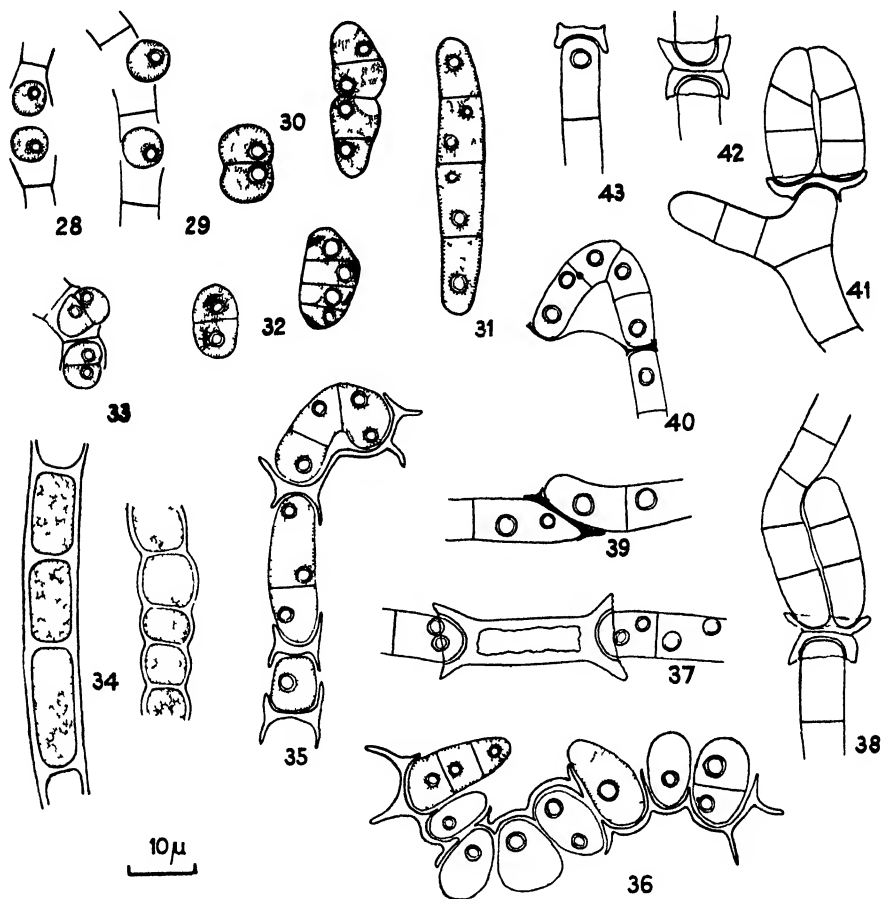
After zoospores have been produced for some little time, aplanospores are commonly formed, although dilution or transference to a new medium results in the recommencement of zoospore-formation. At first an eyespot still appears in the reproductive cells, but no flagella are formed and the contents are liberated as naked motionless cells which germinate like the zoospores. In older cultures no eyespots appear and the contents develop into spherical aplanospores which are liberated in much the same way as zoospores (Figs. 28, 29). They have a thin wall and possess a parietal chloroplast occupying the entire cell and containing a single pyrenoid. They are formed singly or in pairs according to the length of the cell. The terminal cell of the filament to which they give rise remains broadly rounded (Figs. 30, 31) and probably never becomes acuminate, as rounded tips are still seen in filaments of some length. No holdfast is differentiated, the basal cell either remaining unattached or adhering to the bottom of the dish with the help of a little hyaline mucilage. In cultures both the aplanospores and the naked arrested zoospores often form short filaments, whose slightly swollen cells produce zoospores or aplanospores (Fig. 32). Both kinds of structures can also germinate *in situ* (Fig. 33), the young filament growing out through a rupture of the wall. Aplanospores have been recorded in *Uronema* by Lagerheim (1887, p. 521), Chodat (1902, p. 267), and West and West (1903, p. 5), but their further development had not been followed.

No sexual reproduction was observed, but a few biflagellate swimmers were once encountered among numerous four-flagellate zoospores in a liquid culture. They were globular, 6–8 μ in diameter, with a posterior chloroplast having a single pyrenoid and two anterior contractile vacuoles. The elongate eyespot occupied the same position as in the zoospores. The flagella were 1½ times as long as the body (Fig. 27). The further fate of these swimmers could not be followed.

GROWTH OF THE ALGA ON AGAR

On agar the filaments are of indefinite length (30 cm. or more), and as they are much folded in a parallel manner can easily be lifted with a needle. The cells of the younger filaments are elongate and the chloroplast bands are often incomplete. Older threads are constricted at intervals and composed of

shorter cells which are often slightly swollen, sometimes being even broader than long. The chloroplast, which usually has a single pyrenoid, contains abundant starch and fills almost the whole length of the cell. Such cells are in process of zoospore-production. Many of them show an eyespot, while



FIGS 28-43 *Uronema terrestre* n.sp. FIGS 28 and 29 Liberation of aplanospores FIG 30. Stages in germination of aplanospores FIG 31 Filament produced by an aplanospore FIG. 32. Formation of zoospores in young filaments from aplanospores FIG 33 Germination of aplanospores *in situ* FIG 34 Akinetes FIGS 35-43 Germinating akinetes

others develop one within half an hour of being placed in water; zoospores are liberated soon afterwards. Especially in weakly illuminated cultures the entire wall gelatinizes and the incipient zoospores germinate *in situ*. The filament may also break up into one- or two-celled fragments which are capable of growth. In stronger light, the filaments continue to grow indefinitely without much fragmentation. Such filaments show all gradations between an acuminate and a rounded apex (Figs. 21-6).

In old agar or liquid cultures the usually short cells acquire yellowish contents and a slightly thicker wall, while the pyrenoid becomes indistinct. Such cells generally occur in chains which may be separated by dead cells. Similar akinetes have been described in *U. indicum* (Mitra, 1945, p. 115) where they constitute the means of perennation under natural condition. Those of *U. terrestre* can germinate at any stage of development. The contents turn green, the pyrenoid becomes distinct, and division commences.

The ruptured wall persists in various ways around the resulting filaments (Figs. 35-43) and stains deeply with ruthenium red. The parent filament does not immediately break up and the threads formed by the germinating filaments often grow out laterally (Fig. 36). They commonly form loops and, when these break across, two branches appear to arise from the tip of the filament. The filaments formed from the akinetes are slightly broader (7μ). No holdfast is formed and the apex in general remains broadly rounded. Similar features were noted in the germination of the akinetes of *U. indicum* (Mitra, 1945, p. 115). The acuminate apices there recorded probably belonged to filaments originating from zoospores, since those from akinetes produce zoospores at an early stage. This was also so in the case of *U. terrestre* where such filaments formed holdfasts indicating their origin from zoospores.

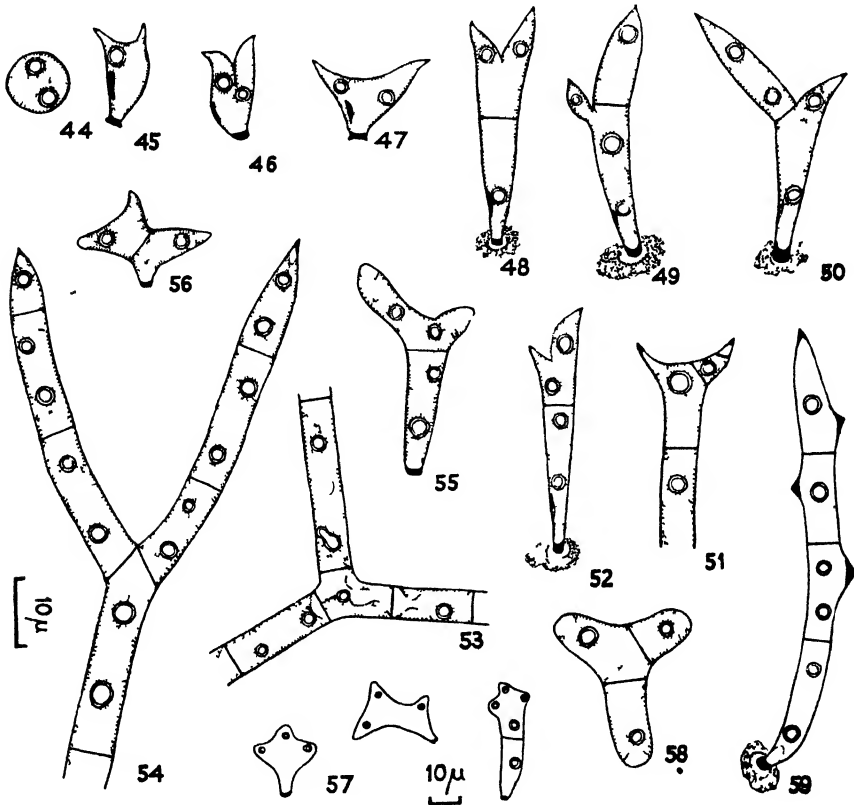
BRANCHING

Branching has been observed in numerous germlings from zoospores and sometimes from aplanospores, both in liquid cultures and on agar. It commences in the one-celled stage with a broadening of the apex and development of two acuminate tips (Fig. 45). These grow into two branches, one or both of which is cut off from the part below, usually by an oblique wall. The branching is thus of the nature of a dichotomy. In no instance are branches formed by lateral outgrowth of intercalary cells. The two branches are generally of the same diameter (Fig. 54) and grow with equal vigour into long unbranched threads, although one of them sometimes lags behind the other and remains short. Such short branches are sometimes not cut off by a septum and then appear as lateral outgrowths (Fig. 49). Occasionally the two branches undergo no further development, but are carried up by segmentation of the part below so that the terminal cell of the filament shows two acuminate tips. In filaments from the bottom of liquid cultures the cells may show pointed thickenings on the side walls (Fig. 59), but it is doubtful if they are of the same nature. Somewhat similar structures are shown by Vischer (1933, figs. 16, 32).

The cells of the branches above described show the usual structure and give rise to zoospores which germinate in the customary manner. The branching is thus not comparable to that observed by Gross (1931, p. 212) in *Ulothrix*, where the branches are described as abnormal and produce zoospores giving rise to abnormal germlings. Moreover, such branching in *Ulothrix* occurs in old cultures, while in *Uronema terrestre* it appears in fresh cultures and is initiated in the young germlings. Lind (1932, p. 713) observed

branching in sporelings of *Ulothrix*, but the origin and fate of these filaments are not known.

Although two branches are the rule in *U. terrestre*, irregular germlings with three or four lobes are sometimes encountered (Figs. 56, 57). Many of these degenerate, but occasionally the lobes grow into branches. By intercalary



FIGS 44-59 *Uronema terrestre* n.sp. Figs 44-54 Branched germlings from liquid cultures Fig 55 Branched germling from agar Figs 56 and 57 Irregular germlings from agar Fig 58 Branched germlings derived from an aplanospore Fig 59 Germling with special thickening of wall

segmentation such branches come to be widely spaced and are never attached at the same point.

With the object of determining the underlying causes the frequency of branching was investigated under various conditions. Material was grown under different conditions of illumination in glass-distilled water, in liquid media with a pH ranging from 11.5 to 2.0 and on solid media. The pH of the medium did not appear to have a marked effect on the incidence of branching, whereas weak illumination and growth on solid substrata appeared to increase it. This was reinvestigated a few months later and the two sets of results are

tabulated below. The estimations were made on the first 200 germlings observed in each case.

PERCENTAGE OF BRANCHED GERMLINGS UNDER DIFFERENT CONDITIONS

Medium.	Month.	Illumination.*	Percentage of branched germlings.
Distilled water	March	Daylight of laboratory	22
	March	Daylight of laboratory	8
	Sept.	Continuous artificial light	3
		Daylight of laboratory	6.5
De's solution	March	Continuous artificial light	2.5
		Daylight of laboratory	9
	Sept.	Continuous artificial light	5
		Daylight of laboratory	7
Agar with De's solution	March	Continuous artificial light	3.5
		Daylight of laboratory	37
	Sept.	Continuous artificial light	9
		Daylight of laboratory	31
		Continuous artificial light	7

*Daylight of the laboratory equal to about $\frac{1}{6}$ and the continuous artificial illumination equal to about $\frac{1}{3}$ of the intensity of normal bright sunlight. The author is indebted to Dr. F. M. Haines for these estimations made with a photo-electric exposure-meter.

Branching is clearly more frequent on agar than in culture solution or distilled water. In the last the germlings begin to degenerate after about a week. Further, branching is more frequent in weak light than in stronger and continuous artificial light. Alternate illumination and darkness at short intervals have no effect on branching. Cultures on agar kept in a greenhouse, or in artificial light for $9\frac{1}{2}$ hours a day, do not show any increase in branching. It thus appears that growth on a solid substratum, coupled with weak illumination, rather than a deficiency of nutriment in the medium is conducive to branching. Weak illumination, of course, reduces photosynthesis. Both Gross (1931, p. 212) and Lind (1932, p. 713) regard branching in *Ulothrix* as due to food deficiency. By placing an agar culture alternately in strong and weak light, at intervals of a week, it is possible greatly to increase the number of branched filaments so that the culture may come to consist largely of such specimens.

TAXONOMIC POSITION OF URONEMA

The following is the diagnosis of *Uronema terrestre*:—Filaments in cultures reaching a length of 30 cm. or more, constricted at intervals and attached by a flat circular disc about 20μ in diameter, with lobed edges and showing brown concentric zones. Cells $3-5\mu$ broad and $22-35\mu$ (sometimes up to 60μ) long, often swollen at the septa. Chloroplast a hollow cylinder, sometimes incomplete, composed of two thickened bands, each containing a pyrenoid, on either side of the middle of the cell and thinner central and terminal portions through which the central vacuole appears as an oval or rounded area in the centre of the cell and as dome-shaped areas at each end adjacent to the septum.

Terminal cell usually shorter, rarely curved, with an acuminate apex. Basal cell tapering at its lower end, of the same length as the other cells and with a well-developed chloroplast. Reproduction by zoospores, aplanospores, and akinetes. Zoospores ($6-15\mu$ long, $5-8\mu$ broad) produced singly or in pairs from any cell, oval to elongate-oval, often with a pointed posterior end, four-flagellate, with two contractile vacuoles at the anterior end and an elongate eyespot usually near the middle and always in contact with the cup-shaped chloroplast, the latter with a single lateral pyrenoid. Germlings usually straight, sometimes branched.

Habitat: In cultures of soils from rice-fields, Allahabad, India.

U. terrestre resembles *U. confervicolum* Lagerh. in its narrow filaments, although those of the latter reach a width of 8μ . Both filaments and cells are longer than those of the latter. Unlike those of *U. confervicolum* the basal cells are as long as the others, contain chloroplasts and can reproduce. The chloroplast of *U. confervicolum* is a parietal plate with a lobed margin and occupies the entire length of the cell, thus differing from that of *U. terrestre*. The attaching disc of the latter differs from the hemispherical holdfast originally described for *U. confervicolum* (Lagerheim, 1887, p. 518), although others have recorded a flat disc for this species. Other distinctive vegetative features of *U. terrestre* are the swollen ends of the cells, the occurrence of thicker septa which results in the formation of H-pieces when the filaments break up and the tendency of the germlings to branch. Specimens from China assigned by Skuja (1937, p. 71) to *U. confervicolum*, however, have filaments constricted at the septa and cells containing a hollow cylindrical chloroplast that fills the whole cell. The position of the eyespot in the zoospores of *U. terrestre* differs from that recorded for *U. confervicolum* (Lagerheim, 1887, p. 519, Chodat, 1902, p. 27) while akinetes have not been reported for the latter species.

U. terrestre resembles *U. gigas* Vischer in possessing very long filaments constricted at intervals, but Vischer's species has much broader and shorter cells, with a chloroplast containing 1-5 pyrenoids. *U. elongatum* Hodgetts and *U. indicum* Ghose are both broader than the species here described, while the chloroplast, which occupies only the middle of their cells, has the form of an incomplete band with incised edges. *U. indicum* is unique in producing multiseriate filaments during reproduction. If Ghose's observations are substantiated in pure cultures of his alga, then, as Fritsch and Rich (1924, p. 319) point out, *U. indicum* should be renamed *Schizomeris indicum*.

The independence of *Uronema* as a genus was first questioned by Gaidukov (1903, p. 524), who observed *Uronema*-like tips in cultures of a species of *Ulothrix*. His view was accepted by Oltmanns (1904, p. 203) and Wille (1909, p. 71), while Fritsch and Rich (1929, p. 34) regarded the absence of the acuminate terminal cell in some of their specimens as supporting evidence. Fritsch (1935, p. 201) also inclines towards this view, while other writers (Collins, 1909; Hodgetts, 1918; Printz, 1929) reserve their judgement. Gaidukov's conclusions have been criticized (Brand, 1913, p. 69) on various grounds.

The end cell of *U. terrestre* when grown on agar shows all gradations from an acuminate to a broadly rounded tip. The acuminate tip, formed by the posterior end of the zoospore, is carried upwards by intercalary growth and once lost is, probably, not re-formed (cf. Fritsch and Rich, 1929, p. 34; Skuja, 1937, p. 71). The other distinctive characteristic, viz. the tapering basal cell ending in an attaching disc, is absent in filaments originating from aplanospores and akinetes. The acuminate tip and the prominent holdfast thus appear to be restricted to germings from zoospores, but are perhaps symptomatic of the normal condition.

The facts point to a close affinity between *Ulothrix* and *Uronema*, but it remains doubtful whether they justify the inclusion of the latter as a subgenus of *Ulothrix*. No indication of the formation of rhizoids was obtained in my numerous cultures. The zoospores, which are perhaps comparable to the macrozoospores of *Ulothrix*, germinate in quite a different way. It therefore seems best to regard *Uronema* as an independent genus closely allied to *Ulothrix*.

Vischer's (1933, p. 74) view that *Uronema* is a reduced member of the Chaetophorales finds little support from the present investigation, although the occurrence of branching might be regarded as of some significance in relation to this hypothesis. The branching of *U. terrestre* is, however, confined to the one-celled stage and is scarcely to be compared to the branching of the Chaetophorales. It seems to represent a special response of the unicellular germling to certain environmental conditions.

SUMMARY

1. A description is given of a new species of *Uronema*, *U. terrestre* n.sp., which appeared in cultures of soils from rice-fields near Allahabad, India, and of its range of variation on soil, in liquid cultures, and on solid media.

2. In cultures it reaches a length of 30 cm. or more. The alga tends to produce zoospores in a large proportion of the cells as soon as there is a slight change in the medium and this results in disintegration of the filament. It is suggested that this probably explains the few-celled filaments usually recorded in *Uronema*.

3. The acuminate tip characteristic of *Uronema* is formed only by germinating zoospores, the posterior portion of which elongates to form the filament while the anterior portion gives rise to the basal cell and the attaching disc.

4. The filaments formed by germinating aplanospores and akinetes do not produce the acuminate tip, nor do they possess the tapering basal cell and attaching disc.

5. On agar the filaments show all gradations between an acuminate and a broadly rounded tip.

6. Branching of a special kind has been observed in germings of zoospores and its frequency under various conditions determined. The branching is of the nature of a dichotomy and takes place only at the one-celled stage. It

is favoured by weak light coupled with growth on a solid substratum rather than by a deficiency of nutriment in the medium.

7. The view is expressed that the genus *Uronema* should for the present be retained.

The author is indebted to Professor F. E. Fritsch for advice and guidance in the course of this investigation.

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A New Method for the Determination of the Composition of the Internal Atmosphere of Fleshy Plant Organs

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With one Figure in the Text

INTRODUCTION

CONSIDERABLE interest has centred around a study of the composition of the internal atmosphere of fleshy plant organs and methods have been described for the extraction of samples of gas for analysis. Probably the earliest work was that by Kidd (1937) carried out in 1916-17. He extracted under vacuum samples of gas from cylindrical plugs cut from the centre of potato tubers, making observations at intervals of a few minutes of the amount of carbon dioxide escaping. He plotted curves of progress for the release of carbon dioxide and found that after a preliminary phase of rapid escape there was a subsequent steady phase, which he attributed to the anaerobic production of carbon dioxide from the metabolism of the tissue. He was able to derive from these curves the total carbon dioxide content of the tissue, i.e. that held in solution or reversible combination as well as that present in the intercellular atmosphere. Curves were then obtained from plugs of tissue which had previously been allowed to attain equilibrium with known concentrations of carbon dioxide in the external atmosphere. He found that comparisons between such plugs and those newly cut could be made from the curves of escape during the first 80 minutes. On the basis of these comparisons he estimated the percentage of carbon dioxide in the internal atmosphere of the potato.

Ekambaram (1922) subsequently employed a more direct method for the estimation of both carbon dioxide and oxygen. This consisted in paring a small area of the peel from the apple and sealing over this area a piece of glass tubing by means of vacuum luting-wax. The lower end of the tube was kept sealed under mercury, and samples of gas were transferred to a Bonnier-Mangin apparatus, an interval being allowed between the taking of successive samples for a state of equilibrium to be reached between the internal atmosphere and the gas content of the glass tube.

Wardlaw and Leonard (1939) inserted into a hole bored in the fruit a sterilized glass tube with a small chamber attached, and sealed it in place by means of a washer and low melting-point wax. They then allowed the gas in the tube and chambers to reach equilibrium with the internal atmosphere of

the fruit and withdrew samples of about 2 ml. volume for analysis in the Haldane apparatus by the dilution method. This method of extraction has more recently been used by Trout et alia (1942).

These methods have the disadvantage that they involve considerable mutilation of the tissues of the fruit and the equilibration of an appreciable volume of gas with the internal atmosphere prior to each analysis. Further, they do not take into account the existence of gaseous diffusion gradients between different parts of the tissue.

The method described here causes a minimum of injury to the tissue and permits sampling to be carried out at relatively short intervals of time.

THE APPARATUS

The essentials of the apparatus employed are shown in the figure. Through a hole in a brass plate (A) a hypodermic needle (B) is passed and soldered in position. The boss of the needle is filed cylindrical and over it is passed a short piece of thick-walled rubber tubing which connects the needle to the shortened delivery end of an inverted, 5-ml., automatic-filling pipette (C). A short piece of wire of suitable gauge is inserted into the capillary of the delivery tube and reduces the dead space in it and in the hollow boss of the needle. The filling port of the pipette is fitted with an S-shaped arm of glass capillary, the lower bend of which is submerged in a mercury trough. The other end of the inverted pipette is joined by rubber tubing to the lower end of a reservoir (D), consisting of 10 cm. of 26-mm. glass tubing. The upper end of the reservoir is filled with water and connected to an aspirator bottle, sufficient mercury having been introduced into the pipette and reservoir to ensure that when the aspirator bottle has emptied the level of mercury will have fallen enough to suck in the required volume of gas through the needle. If desired one aspirator can serve several sets of apparatus.

The fruit must be mounted before the needle is attached to the pipette. A small square of thin rubber sheet is first sealed on to its surface by means of low melting-point paraffin wax. This prevents the needle tearing the tissue at the point of insertion, and ensures a gas-tight seal. In order to prevent blocking of the needle a wire filament must be inserted so that its end just shows at the tip. The apple is then pressed down on to the needle until it rests upon a cushion (E) formed by making a circle of small-gauge thick-walled rubber tubing and joining the ends together by means of a piece of glass rod. The filament is then withdrawn and the boss of the needle is connected to the pipette.

When it is desired to control the composition of the external atmosphere round the fruit, or when simultaneous measurement of the rate of production of carbon dioxide is required, a flanged chamber (F) with an outlet tube may be sealed to the plate which is provided with a metal inlet tube.

At the commencement of sampling the delivery-tube is filled with mercury, the tap turned to connect the pipette with the needle, and the level of mercury in the pipette is adjusted so that the bore of the tap is just filled

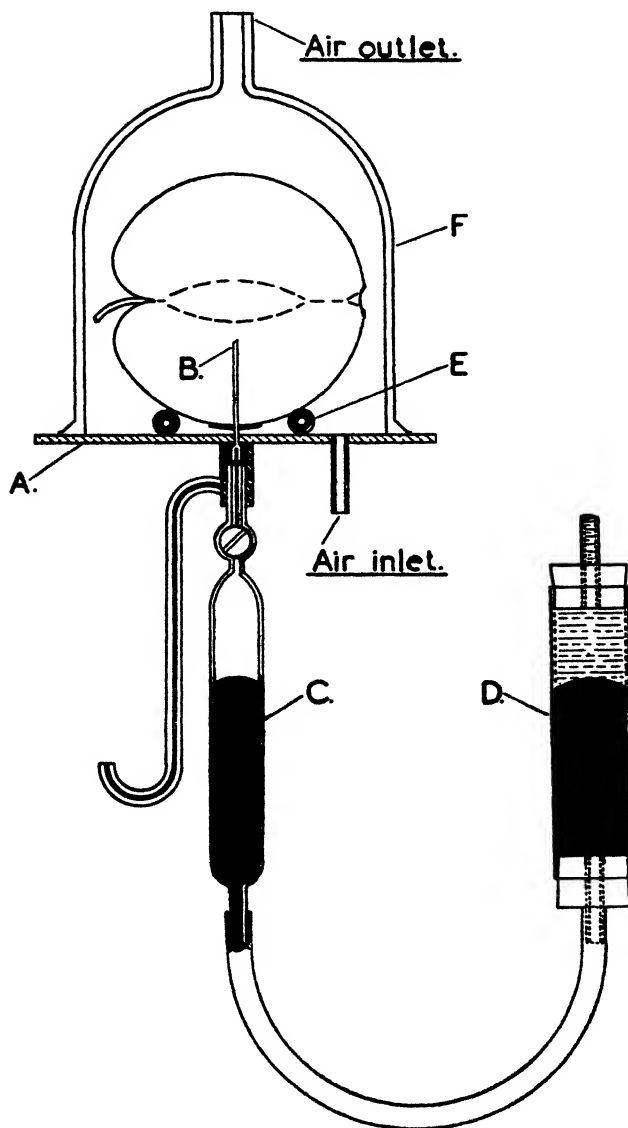


Figure showing details of apparatus. For description see text.

with mercury. The mercury is then allowed to fall sufficiently to draw a sample of the required size into the pipette, the time taken to withdraw the sample being determined by the rate of drip from the aspirator bottle.

When the sample has been collected in the pipette, the tap is turned off and the aspirator bottle refilled. An inverted mercury-filled receiver is held over the end of the delivery-tube submerged in the trough and the sample is discharged into the receiver for analysis.

In testing the method, samples were analysed in the Bonnier-Mangin micro-gas-analysis apparatus, each sample being of approximately 0.5 ml. in volume. Alternatively where it is deemed feasible to withdraw samples as large as 2 ml. the delivery arm of the pipette could be connected to the Haldane gas-analysis apparatus employing a 10-ml. burette, the sample being diluted with nitrogen and the analysis carried out in the usual way.

Analyses made in this manner have proved consistent and the examples given below illustrate the employment of the apparatus in studying the internal carbon-dioxide- and oxygen-content of the apple.

THE MEASUREMENT OF INTERNAL GAS CONCENTRATION

Duration of the period of extraction

In Table I is given a short series of analyses made over a period of five days, employing different extraction periods. It can be seen that no appreciable difference was observed in the composition of the internal atmosphere attributable to duration of the period of extraction, which was varied from 1 minute to 16 hours.

TABLE I

Internal Atmosphere and Variation in Length of Extraction Period

Apple: Cox's Orange Pippin, imported from New Zealand.
Approximate composition of external atmosphere, CO₂ 5%,
O₂ 2.5%, N₂ 92.5. Constant temperature 4.4° C.

Day.	Extraction period.	Carbon dioxide (per cent.)	Oxygen (per cent.)	Nitrogen*
†8	8 hours	6.1	0.6	93.3
9	16 "	6.2	0.7	93.1
9	1 minute	6.3	0.8	92.9
12	8 hours	6.4	0.7	92.9
13	16 "	6.1	0.8	93.1
13	4 "	6.2	0.8	93.0
13	1 hour	6.3	0.8	92.9

* By difference.

† Needle inserted at day 0.

Location in the flesh of the point of extraction

Three needles of length ranging from 0.7 to 2.5 cm. were inserted in the pattern of a triangle of sides 1 cm. at a point on the surface of an apple half-way between stem- and calyx-end and samples of gas were withdrawn from the different depths of penetration. It was found that the composition of the gases varied, the carbon dioxide directly with the depth of penetration and

the oxygen inversely. This is shown by Tables II and III, in which the results of analyses for carbon dioxide, oxygen, and nitrogen (estimated by difference) are recorded for each position in two apples. That this is a real gradient of concentration and is not due to dilution by replacement with incoming air is evident from the following considerations. Taking a figure of 35 per cent. as the average volume of internal atmosphere in a Lord Derby apple (Smith, 1937), the replacement volume is only about one-fortieth of this total. Moreover, the incoming gas entered the apple over a period of 16 hours, allowing ample time for equilibration between tissue fluids and internal atmosphere.

TABLE II

Location of Point of Extraction in Relation to Composition of Internal Atmosphere

Apple: Lord Derby: radius of fruit 3.2 cm., wt. 128.5 gm.

Location: position 1, in equatorial plane, 0.7 cm. beneath skin; position 2, in equatorial plane, 1.3 cm. beneath skin; position 3, in equatorial plane, 2.5 cm. beneath skin.

Constant temperature 12° C. Composition of external atmosphere: day 0-23, air; day 23-37, gas mixture of average composition, CO₂ 10%, O₂ 8.9%, N₂ 81.1%; day 37-48, air.

Day	Composition of internal atmosphere								
	Carbon dioxide (%)			Oxygen (%)			Nitrogen* (%)		
	1	2	3	1	2	3	1	2	3
†4	0.9	—	—	19.6	—	—	79.5	—	—
5	—	—	1.8	—	—	18.1	—	—	80.1
6	—	1.2	—	—	19.0	—	—	79.8	—
7	—	—	1.8	—	—	18.2	—	—	80.0
15	1.4	—	—	18.8	—	—	79.8	—	—
18	—	1.5	—	—	18.9	—	—	79.6	—
21	—	—	1.8	—	—	17.5	—	—	80.7
22	1.3	—	—	19.4	—	—	79.3	—	—
23	—	1.6	—	—	18.2	—	—	80.2	—
Mean	1.2	1.4	1.8	19.3	18.7	18.3	79.5	79.9	80.3
27	—	10.3	10.8	—	7.9	8.1	—	81.8	81.1
28	10.3	—	—	8.3	—	—	81.4	—	—
29	—	—	10.8	—	—	8.0	—	—	81.2
31	—	10.4	—	—	8.1	—	—	81.5	—
33	10.8	—	—	7.8	—	—	81.4	—	—
34	—	—	10.8	—	—	7.7	—	—	81.5
35	—	10.8	—	—	7.4	—	—	81.8	—
36	10.6	—	—	7.6	—	—	81.8	—	—
37	—	—	10.9	—	—	7.3	—	—	81.8
Mean	10.6	10.5	10.8	7.9	7.8	7.8	81.5	81.7	81.4
40	2.4	—	—	17.2	—	—	80.4	—	—
41	—	2.8	—	—	17.0	—	—	80.2	—
42	—	—	3.4	—	—	16.6	—	—	80.0
46	2.5	—	—	17.0	—	—	80.5	—	—
47	—	2.7	—	—	16.9	—	—	80.4	—
48	—	—	3.0	—	—	16.7	—	—	80.3
Mean	2.4	2.8	3.2	17.1	17.0	16.6	80.4	80.3	80.2

* By difference.

† Needle inserted at day 0 (Nov. 11, 1939).

TABLE III

Location of Point of Extraction in Relation to Composition of Internal Atmosphere

Apple: Cox's Orange Pippin imported (New Zealand): radius of fruit 3.5 cm.

Location: position 1, in equatorial plane, 0.8 cm. beneath skin; position 2, in equatorial plane, 1.6 cm. beneath skin; position 3, in equatorial plane, 2.4 cm. beneath skin.

Constant temperature 4.4° C. Composition of external atmosphere: day 7-14, air: day 14-25, gas mixture of average composition, CO₂ 5.0%, O₂ 2.2%, N₂ 92.8%.

Day	Composition of internal atmosphere								
	Carbon dioxide (%)			Oxygen (%)			Nitrogen* (%)		
	1	2	3	1	2	3	1	2	3
†7	—	2.0	—	—	18.6	—	—	79.4	—
13	—	—	2.6	—	—	18.2	—	—	79.2
14	1.8	2.1	—	18.8	18.7	—	79.4	79.2	—
Mean	1.8	2.0	2.6	18.8	18.6	18.2	79.4	79.3	79.2
19	—	—	6.0	—	—	1.4	—	—	92.6
20	—	5.7	—	—	1.4	—	—	92.9	—
21	5.5	—	6.2	1.8	—	1.4	92.7	—	92.4
22	—	5.9	—	—	1.5	—	—	92.6	—
23	—	—	5.9	—	—	1.1	—	—	93.0
25	—	5.8	—	—	1.5	—	—	92.7	—
Mean	5.5	5.8	6.0	1.8	1.4	1.3	92.7	92.7	92.7

* By difference.

† Needle inserted at day 0 = March 13, 1940.

SUMMARY

A method is described for extracting from the internal atmosphere of the apple small samples of gas for analysis by the Bonnier-Mangin technique. Tests are reported indicating the consistency of the results obtained and the existence of a gradient of gas concentration between the inner and outer parts of the flesh.

The author wishes to acknowledge the assistance of Mr. D. N. Rhodes who carried out the manipulations and analyses required for the exploration of this method.

The work described above was carried out as part of the programme of the Food Investigation Board of the Department of Scientific and Industrial Research.

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The Persistence of a Nucleolar Remnant during Meiosis in a Diploid Banana

BY

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AND

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With Plate II and nine Figures in the Text

1. INTRODUCTION

THE following note describes a peculiar behaviour of the nucleolus that has been observed during male meiosis in a seeded diploid of *Musa* ($x = 11$). The clone under consideration was grown from seed received from Assam and has been arbitrarily named Mariani (Accession number, I.R. 209); its phenotype suggests affinity with *Musa acuminata* Colla.

Preparations were temporary squashes of pollen mother-cells prefixed in acetic-alcohol and stained in acetocarmine. Root tips were fixed in Craib and stained in iodine crystal-violet.

2. THE SOMATIC NUCLEOLUS

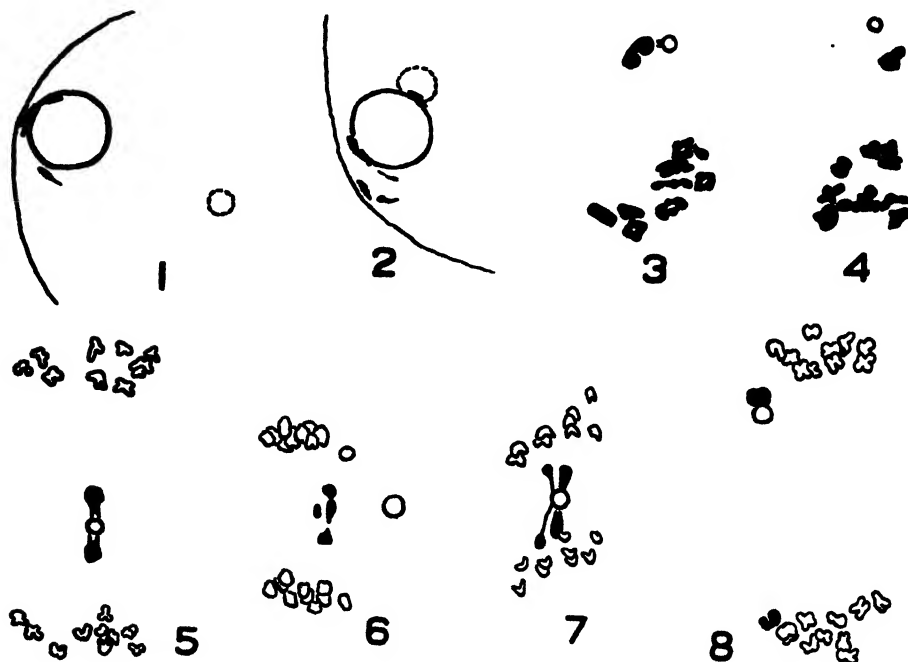
In the somatic nucleus there are two nucleolar organizers that are usually attached to a single nucleolus, for their separate nucleoli fail to fuse in only about 5 per cent. of cells. Thus in 500 resting cells of the root-tip meristem, 473 nuclei had one nucleolus and 27 had two.

3. THE MEIOTIC NUCLEOLUS

The early sequence of prophase cannot be interpreted, but the large nucleolus decreases in size while still attached to a stained body, presumably the nucleolar organizer. A lightly stained nucleolar bud, free or attached to the parent nucleolus, is also present (Text-figs. 1 and 2), but this disappears later in prophase.

At pro-metaphase there is a nucleolar remnant. This sometimes lies free in the cytoplasm and is then clearly visible as a small lightly stained sphere about the same size as a daughter bivalent (Pl. II, Fig. 2, and Text-fig. 4). In these cells it persists through later stages and is included in the cytoplasm of one of the daughter cells. Usually, however, the remnant remains attached to the nucleolar bivalent and, as a result, may delay its congression at metaphase (Text-fig. 3; cf. Pl. II, Fig. 1). When attached, the remnant is often difficult to identify, and this probably explains its apparent absence in many

cells at full metaphase—it is obscured by the chromosomes. Subsequently various behaviours may occur. Quite commonly the remnant passes into one of the polar groups still attached to a daughter bivalent, the movement of which is often delayed (Text-fig. 8). Sometimes it is left on the equator (Text-fig. 6), and in these cells it may have been unattached previously or have lost connexion with the separating daughter bivalents. Occasionally



TEXT-FIGS. 1-8. Figs. 1 and 2. Parts of two early mid-prophase nuclei showing the nucleolus, organizer, and nucleolar bud (dotted outline). Fig. 3. Prometaphase, showing the nucleolar remnant attached to and delaying the congression of the nucleolar bivalent (which, in drawing, is adjusted nearer plate). Fig. 4. Prometaphase showing a detached nucleolar remnant. Fig. 5. A nucleolar bridge at first anaphase. Fig. 6. Bridge and fragment at first anaphase, the nucleolar remnant lagging by the equator. Fig. 7. Double nucleolar bridge. Fig. 8. Carriage of the nucleolar remnant to the pole by one of the nucleolar daughter bivalents. ($\times 1,500$.)

connexion with the undisjoined bivalent is retained and, as a result, a bridge is formed (Text-fig. 5; Pl. II, Fig. 3). Of sixteen bridges of nucleolar origin, four were double, with two bridges attached to the nucleolar remnant (Text-fig. 7). The double complex probably results from the coincidence in one cell of a nucleolar and a non-nucleolar chromatid bridge, the latter being either accidentally attached to a 'sticky' nucleolar surface or collapsed upon it as a result of fixation. Simple chromatid bridges, arising presumably from crossing-over within a small inversion, occur rarely (cf. Dodds and Simmonds, *in the press*); bridges with fragments are also to be seen, though again only rarely (Text-fig. 6). Nucleolar bridges persist at late anaphase and telophase;

they may be relatively unextended (Pl. II, Fig. 4), but usually are greatly stretched so that the centric regions near or even in the polar groups are attached by long strands to the nucleolar remnant still in an equatorial position.

It is unlikely that the remnant is often obscured by the anaphase chromosomes. Anaphases, therefore, in which it is not found, may be confidently interpreted as denoting its true absence.

At interphase the remnant is sometimes seen in the cytoplasm of one of the daughter cells, but in most diads it is not recognizable, being either truly absent or included in one of the interphase nuclei.

The identification of the remnant at the second division is somewhat easier than at the first, owing to the smaller size of the chromosomal bodies and the freer spacing of the plate. But again it may have been obscured by the chromosomes in a few cells, although this probably occurs even less frequently at anaphase than at metaphase. Behaviours are similar to those of the first division except that no bridges are seen.

4. A QUANTITATIVE DESCRIPTION OF NUCLEOLAR BEHAVIOUR

Data on the frequency of the various nucleolar behaviours at successive stages of meiosis are given in Table I; they are collated from fixations of

TABLE I

Frequencies of Various Nucleolar Behaviours at Successive Stages of Meiosis

	Number of cells with nucleolus					
Meiotic stage.	Attached.		Unattached.		Not found.	Total.
First metaphase	6		28		16	50
	Attached.		Unattached.			
First anaphase and telophase	In polar group.	In bridge.	Lagging.	Not con-gressed.		
	35	16	19	98	15	183
Interphase	0			51	49	100
Second metaphase	2			41	41	84
	Attached.		Unattached.			
Second anaphase and telophase	In polar group.	In bridge.	Lagging.	Not con-gressed.		
	2	0	1	12	15	30

several male buds at different times. The nucleolus is considered as 'attached' when joined to a chromosome, 'unattached' when free in the cytoplasm, and 'not found' when apparently absent. The second category represents an accurate score, for, as pointed out in the preceding description, the nucleolar remnant can be clearly recognized when free in the cytoplasm. But between the two other categories there is a reciprocal error arising from the fact that some cells in which the nucleolar remnant is attached are unavoidably scored as 'not found'. This error is considerable at first metaphase and interphase, smaller but appreciable at second metaphase, and negligible at both anaphases.

Thus in Table II the data (as percentages) are transformed by corresponding, but necessarily arbitrary, adjustments for the error, and the 'not found' category now represents cells in which the remnant has disintegrated. The

TABLE II
Observed and Adjusted Percentages (Brackets) of Cells showing Various Nucleolar Behaviours

Meiotic stage.	Nucleolus			Adjustment.
	Attached.	Unattached.	Disintegrated.	
Prophase	100 (100)	0 (0)	0 (0)	None
First metaphase	12 (42)	56 (56)	32 (2)	30% 'disintegrated' to 'attached'
First anaphase and telophase	28 (28)	64 (64)	8 (8)	None
Interphase	0 (20)	51 (51)	49 (29)	20% 'disintegrated' to 'attached'
Second metaphase	2 (12)	49 (49)	49 (39)	10% 'disintegrated' to 'attached'
Second anaphase and telophase	7 (7)	43 (43)	50 (50)	None

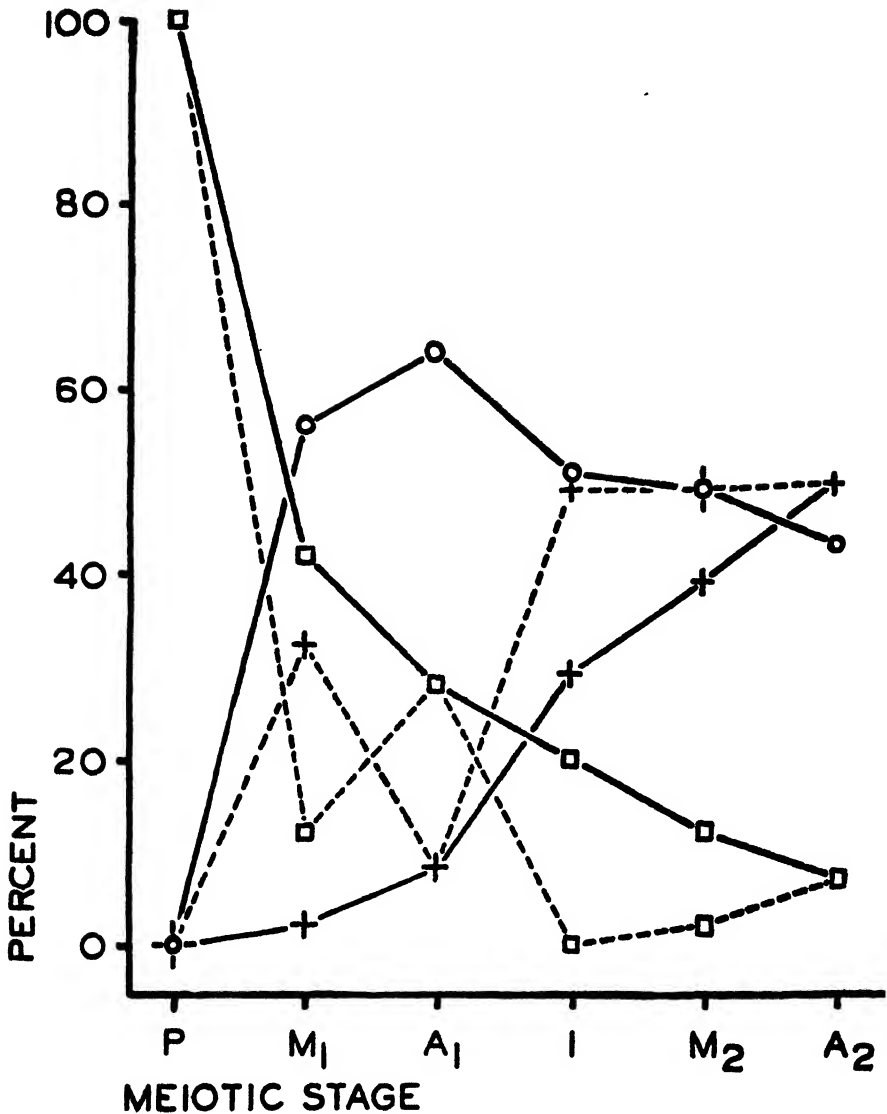
observed and adjusted percentages are shown graphically in Text-fig. 9, wherein it will be seen that the relative courses of the unadjusted 'attached' and 'not found' curves substantiate the presence of the reciprocal error inferred from cytological observation. The two processes of detachment and loss appear to proceed concurrently with the former predominating until the end of meiosis, by which time the 'attached' category declines to less than 10 per cent. Evidently detachment is a normal preliminary to disintegration of the nucleolar remnant, although this conclusion obviously does not exclude the possibility that disintegration may sometimes occur while the remnant is still attached to the chromosomes. The sequence may be summarized thus:



The interest of these observations lies in the fact that the nucleolar behaviour has mechanical consequences for the meiotic chromosomes comparable in type but different in nature from those observed by Darlington (1935).

5. SUMMARY

A seeded diploid *Musa* of uncertain specific status shows a persistent nucleolar remnant in male meiosis. The remnant usually becomes detached from its organizing chromosomes during the course of meiosis and, once detached, commonly disintegrates. It sometimes causes poor congression of the nucleolar bivalent at metaphase and lagging at first anaphase to form a bridge; the behaviour is thus an example of interference by the nucleolus in



TEXT-FIG. 9. The behaviour of the nucleolar remnant through the course of meiosis in a diploid banana (Mariani). (Squares = 'attached'; circles = 'unattached'; crosses = 'dis-integrated'. Solid lines = adjusted values; dotted lines = unadjusted. P, prophase; M₁, first metaphase; A₁, first anaphase and telophase; I, interphase; M₂, second metaphase; A₂, second anaphase and telophase. See Table II and Text.)

the mechanics of meiosis. Occasional double nucleolar bridges at first anaphase probably indicate that bridges arising from structural hybridity of the chromosomes may become entrapped upon the nucleolar surface.

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DESCRIPTION OF PLATE II

Illustrating N. W. Simmonds and K. S. Dodds article, 'The Persistence of a Nucleolar Remnant during Meiosis in a Diploid Banana'.

All figures magnified c. 1,000.

Fig. 1. First metaphase with an attached nucleolar remnant.

Fig. 2. First metaphase with the nucleolar remnant free in the cytoplasm (at 4 o'clock from the plate).

Fig. 3. Nucleolar bridge at first anaphase.

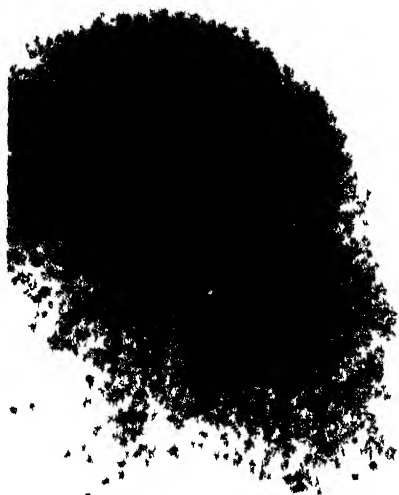
Fig. 4. Nucleolar bridge persisting at first telophase.



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Comparative Physiological Studies on the Growth of Field Crops

II. The Effect of Varying Nutrient Supply on Net Assimilation Rate and Leaf Area

BY

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With five Figures in the Text

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INTRODUCTION

IN a previous paper (Watson, 1947) an analysis of the variation in dry-matter production by different species and varieties of field crops during six growing seasons was made in terms of net assimilation rate and leaf area. Net assimilation rate, the rate of increase of dry-matter per unit area of leaf, was shown to vary with time throughout the year, and to differ at comparable calendar periods of different years. Differences in net assimilation rate between species, compared in the same calendar period, and in some cases between varieties, were also demonstrated. However, the variation in net assimilation rate between years was small compared with the variation in leaf area. Differences in dry-matter yield between species were found to be closely related to differences in leaf-area duration, the integral of leaf area per unit area of crop over the period of growth. The mean net assimilation rate, weighted at any time by the magnitude of leaf area at that time, was found to be almost constant for the four species investigated. This was because the species with the higher net assimilation rate, sugar-beet and potatoes, had a small leaf area in June and July at the time when net assimilation rate reached its maximum in the seasonal cycle, and did not develop a large leaf area until late in the season when net assimilation rate had fallen to low values, while in wheat and barley, the species with lower net assimilation rate, maximal leaf area occurred near to the time of the seasonal peak in net assimilation rate. Variation in

dry-matter accumulation between years, and between species and varieties, was therefore determined mainly by differences in the size and duration of the assimilating system, as measured by leaf area, and variation in net assimilation rate was of minor importance.

In the present paper a similar analysis is made of differences in dry-matter yield of field crops produced by variation in nutrition. For this purpose it was decided to make use of material taken from three of the classical field experiments established at Rothamsted by Lawes and Gilbert, namely, Broadbalk field (continuous wheat since 1843), Hoosfield (continuous barley since 1852), and Barnfield (continuous mangolds since 1876). In each of these experiments the same crop has been grown for many years on plots receiving varied annual fertilizer treatments, which have continued unchanged or with only minor alterations throughout the period.

The reason for this choice of material was that severe nutrient deficiency has developed on the plots where nutrients have been withheld, so that the differences between plots in nutrient supply and the responses in crop growth and yield are now much greater than could be produced by varied fertilizer dressings applied to any of the ordinary arable fields at Rothamsted. As the necessity for handling large samples of plant material limits the accuracy with which growth attributes can be estimated in the field, it is obviously advantageous to use material in which the differences of dry-matter yield produced by varying nutrient supply are as large as possible.

One disadvantage of using these experiments is that as the treatments are arranged systematically and are not replicated, it is not possible to compute valid estimates of error of the observed treatment effects. The factorial arrangement of the treatment provides some degree of internal replication, and the effects to be discussed are based on means of several plots. As the treatments are not allotted to plots at random, the estimates of the effects involve contrasts between different positions in the field, and are biased to an unknown extent by differences in soil fertility. The results must therefore be interpreted with caution.

MATERIAL AND METHODS

The work described in this paper was carried out in 1939. A full account of the early history of the experiments is given by Hall (1905) and of the more recent results by Russell and Watson (1938, 1939, 1940) and Watson and Russell (1943, 1945, 1946). It is unnecessary to describe the design of the experiments in detail here; the plots from which plant material was taken, and their annual treatments, are shown in Table I. It should be noted that the rates of application of fertilizers differ in the three experiments. Also, on Barnfield sodium is supplied as chloride, while on Broadbalk and Hoosfield sulphate is used. In all three experiments sodium and magnesium salts are given together, but it is probable that the effects of the mixture are mainly attributable to its sodium content; the amounts of magnesium supplied are comparatively small.

Of the seven plots selected from Broadbalk, four receive treatments consisting of all combinations of the presence and absence of the two factors, nitrogen and a complete mixture of mineral fertilizers (phosphate, potassium,

TABLE I

Annual Manurial Treatments of the Selected Plots

Wheat was Red Standard sown on October 25, 1938; Barley was Plumage-Archer sown on March 10, 1939; Mangold was Yellow Globe sown on May 9, 1939.

Wheat, Broadbalk.		Barley, Hoosfield.			Mangolds, Barnfield.		
		Series O.		Series A.	Series O.		Series A.
Plot.*	Treatment.	Strip.	Treatment.		Strip.	Treatment.	
2	d	1	(1)	n	1	d	dn
3	(1)	2	p	np	2	dpk	dnpk
5	pks	3	ks	nks	4	pks	npks
7	npks	4	pks	npks	5	p	np
10	n	Plot.			6	pk	npk
11	np	6-1	(1)		7	ps	nps
13	npk	7-2	d		8	(1)	n

* Samples were taken from two sections of each plot: section V, following fallow in 1938, and section III, following three years' cropping after fallow in 1935.

Key to treatment symbols. (1) = unmanured. d = 14 tons farm-yard manure (200 lb. N, 100 lb. P_2O_5 , 250 lb. K_2O) per acre. n = 412 lb. sulphate of ammonia (86 lb. N) per acre, Broadbalk and Barnfield; 206 lb. sulphate of ammonia (43 lb. N) per acre, Hoosfield. p = 3½ cwt. superphosphate (70 lb. P_2O_5) per acre. k = 200 lb. sulphate of potash (100 lb. K_2O) per acre, Broadbalk and Hoosfield; 500 lb. sulphate of potash (250 lb. K_2O) per acre, Barnfield. s = 100 lb. sulphate of soda (20 lb. Na_2O), and 100 lb. sulphate of magnesia (17 lb. MgO) per acre, Broadbalk and Hoosfield; 200 lb. agricultural salt (sodium chloride) (100 lb. Na_2O) and 200 lb. sulphate of magnesia (35 lb. MgO) per acre, Barnfield. The weights per acre of N, P_2O_5 , K_2O , Na_2O , and MgO given in brackets are rough approximations.

All the fertilizers are applied in the seed-bed, except that on Broadbalk, one-quarter of the nitrogen, and on Barnfield, one-third of the nitrogen, is given in the seed-bed, and the remainder as a top-dressing.

The cropping and treatment of the Broadbalk and Hoosfield plots has continued practically unchanged since 1852, and that of the Barnfield plots since 1876, except that, up to 1895, strip 2 received only farm-yard manure and superphosphate, and before 1903 strip 7 received the same treatment as strip 6, with the addition of a very small dressing of ammonium salts.

sodium, and magnesium salts). The other three plots measure the effect of farm-yard manure, and of successive additions of phosphate and potash, in the presence of nitrogen. Phosphate is tested only in the absence of potash, and no information is provided on the interaction of potassium and sodium.

The selected plots of Hoosfield form a complete factorial set of treatments, testing all combinations of the presence and absence of the three factors, nitrogen, phosphate, and the mixture of potassium, sodium, and magnesium salts. In this experiment no separation of the effects of potassium and sodium is possible. Two other plots, 6-1 and 7-2, additional to the main series, show the effect of farm-yard manure application.

The Barnfield plots can be divided into three factorial groups which, respectively, include all combinations of the following factors: (1) farm-yard manure,

nitrogen, phosphate, and potash applied together; (2) nitrogen, potash, sodium, and magnesium salts applied together (all tested in the presence of phosphate); (3) nitrogen, phosphate. Six of the plots (5, 6, and 8 of series O and A) each appear in two of the three groups. As on Broadbalk, the effect of phosphate is tested only in the absence of potassium and sodium salts. The factorial groups of plots are set out in Table II.

Although each of the experiments includes comparisons between three forms of nitrogenous fertilizer, sulphate of ammonia, nitrate of soda, and rape-cake, only the sulphate of ammonia plots and the plots receiving no nitrogenous fertilizer were used, in order to reduce the labour of sampling. The sulphate of ammonia series was selected in preference to the nitrate of soda series because of the complication introduced by the supply of sodium in the nitrate of soda.

A small part of each of the selected plots was set apart to provide the samples of plant material. It would have been preferable to distribute the samples over the whole of each plot, but this was not possible, for it was necessary to preserve the greater part for the estimation of yield.

The plots on Broadbalk consist of strips running the length of the field from east to west. Since 1931 the field has been divided transversely from north to south into five sections, each including one-fifth of every plot, which are fallowed in successive years to control weeds. Samples were taken from the west end of section V (fallowed the previous year) and from the east end of section III (the fourth crop after fallow in 1935); these areas were separated by section IV, fallowed in 1939. Thus the data provide information on the effects of fallow as well as fertilizer application.

Each sample consisted of a length of $\frac{1}{3}$ metre of drill-row taken from six neighbouring rows, two metre-lengths of drill-row in all. The section of each plot set apart for sampling was divided into four parts. At each time of observation one sample was taken at random from each quarter; the total amount of crop taken per plot on each sampling occasion was therefore eight metre lengths of drill-row. Samples were separated from each other by unsampled margins of crop, so that the removal of a sample should not affect the subsequent growth of its neighbours.

A similar procedure was followed on the barley plots of Hoosfield, except that the sampling area of each plot was divided into five parts, ten metre lengths of row being taken from each plot at each time of sampling. In this experiment the sampled areas lay at the east end of series A (plots receiving sulphate of ammonia) and the west end of series O (plots receiving no nitrogenous fertilizer), on either side of a narrow path, in a compact strip running across the experiment.

On Barnfield the area available for sampling was too small to allow of random selection because the provision of margins between samples would have meant that a large fraction of the crop would have been wasted, owing to the wide spacing of the plants. A systematic method of sampling was therefore adopted. On the first occasion 10 plants were taken at the end of

each of the two edge rows of a plot, 20 plants per plot in all, the end plants of the rows being rejected. On the next occasion 10 plants were taken from each of the two rows next to the edge rows, and so on for subsequent occasions until at the last sampling the two centre rows of a plot were taken. Thus the plants taken at any time had grown since the previous sampling without competition from neighbouring plants on one side of them. The effect of this reduction in competition in the interval between samplings on the growth of the plants was probably not serious, because the intervals were short, the spacing of the rows wide (26 in.), and all the samples taken from different plots and on different occasions were comparable in this respect. Time trends in the attributes measured may have been affected to some extent because the removal of neighbouring plants is likely to have had a greater effect in the later stages of growth when competition is intense than in the early stages when there is little or no competition.

The samples of cereals were based on unit area of crop, as the spacing between rows was constant (6 in.), while each sample of mangolds consisted of a fixed number of plants, the area per sample varying with the spacing between plants in the row. The reason for this difference was that, as soon as tillering starts, it is difficult or impossible to distinguish individual plants in a cereal crop, while if the size of a sample of a widely spaced crop, such as mangolds, is defined by area, it is frequently doubtful whether a plant should be included in a sample or not, and this may lead to serious errors when the area covered by the sample is small.

Samples were taken from the wheat plots at fortnightly intervals between late April and the time of ear emergence in June, except that the last two intervals were each only a week. For barley, weekly intervals were used throughout, covering the period from mid-May to early July. Ear emergence was nearly completed by the last sampling occasion. The sampling of the mangolds began in July, immediately after the plants were singled, and continued at fortnightly intervals until the middle of October.

The observations made on the samples have been described previously (Watson, 1947); the primary data used in this paper consist of records of the following attributes, made on each sampling occasion: (1) total dry-weight per sample, (2) leaf area per sample, (3) shoot number per sample for the cereals, and leaf number per sample for the mangolds.

Net assimilation rate (N A R) was computed from (1) and (2) according to the formula

$$\frac{W_2 - W_1}{t_2 - t_1} \times \frac{\log_e L_2 - \log_e L_1}{L_2 - L_1}$$

where W_1 and W_2 are the total dry-weights per sample and L_1 and L_2 the leaf areas per sample, at times t_1 and t_2 respectively. N A R is expressed in gm. per sq. metre of leaf area per week, instead of the usual units of gm. per sq. dm. per week, to simplify the printing of the tables.

The results are presented in the form of tables which show values for each treatment, and also for the main effects and interactions of the factors in each

factorial group. The notation and method of estimation of interactions are those described by Yates (1937). Small letters are used to specify treatments, and capital letters for effects and interactions. Thus, the symbol *npk* represents the treatment consisting of the simultaneous application of nitrogen-phosphate, and potash, while the symbol N.PK denotes the interaction between nitrogen and the dressing of phosphate and potash applied together. A main effect, or average response to a factor, is measured by the differences between the mean for all plots where the factor is present and the mean for all plots where it is absent. First-order interactions are estimated as half the difference between the mean responses to one factor in the presence and absence of the other. Similarly second-order interactions are estimated as half the difference between the interactions of two factors in the presence and absence of the third. The main advantage of this method of presentation is that the estimates of main effects and interactions have the same standard errors. The relative importance of main effects and interactions in contributing to the total variation can readily be seen from the tables, for the variance attributable to main effects and interactions are proportional to the squares of the tabulated values. As already noted, no valid test of the significance of the treatment effects is possible because of the systematic arrangement of the treatments and the lack of replication. The significance of the main effects can be judged roughly by using the interactions as approximate estimates of error; if the main effect of a factor is large compared with its interactions, it is justifiable to conclude that the effect is real and not due to chance.

RESULTS

The changes with time in mean N A R and mean leaf area for all treatments have already been discussed (Watson, 1947). The following account is concerned only with the effects of treatments, and the time trends of these effects.

1. *Dry-matter accumulation*

The effect of variation in nutrient supply on increase in dry-weight between the first and last observations on each crop is shown in Table II. The proportion of the final dry-weight accumulated during the period of observation was about 70 per cent. for wheat, 90 per cent. for barley, and over 98 per cent. for mangolds. Consequently the results shown in Table II correspond closely with the effects on the yields of the crops at harvest, which are well known and have been described in the accounts of the experiments already cited. A detailed discussion of Table II is therefore unnecessary; it is sufficient to mention the more important effects and to point out a number of ways in which the results for the 1939 season differed from those usually observed.

Farm-yard manure, nitrogenous fertilizer, and fallow all caused large increases of dry-matter production in wheat. The nitrogen effect was greater in the presence of the complete mineral fertilizer treatment (*pks*) than in its absence. The reduction caused by phosphate (plots 10 and 11) has been a regular feature in recent years, though it did not occur in the period before

TABLE II

Dry-matter Accumulation during the Experimental Period

WHEAT. Increase of dry weight (gm. per metre) between April 25 and June 20

Plot.	Treatment.	After crop.	After fallow.	Mean.
2	d	97	138	118
3	(1)	29	61	45
5	pks	25	52	39
7	npks	93	121	107
10	n	83	54	68
11	np	55	60	57
13	npk	63	79	71
	Mean	64	81	72

BARLEY. Increase of dry weight (gm. per metre) between May 16 and July 7.

Plot.	Treatment.		Effect.	
1-0	(1)	34	Mean	58.6
2-0	p	40	P	11.6
3-0	ks	37	KS	6.2
4-0	pks	39	P.KS	-0.2
1-A	n	66	N	42.4
2-A	np	83	N.P	7.4
3-A	nks	75	N.KS	4.7
4-A	npks	96	N.P.KS	2.0
6-1	(1)	32		
7-2	d	112	D	80.3

MANGOLDS. Increase of dry weight (gm. per plant) between July 14 and October 18.

Plot.	Treatment.		Effect.	
8-0	(1)	13	Mean	104.2
6-0	pk	29	PK	43.4
1-0	d	65	D	84.8
2-0	dpk	108	D.PK	4.9
8-A	n	72	N	101.1
6-A	npk	133	N.PK	14.0
1-A	dn	180	D.N	19.6
2-A	dnpk	234	D.N.PK	-8.7
5-0	p	19	Mean *	70.0
6-0	pk	29	K	5.6
7-0	ps	37	S	6.0
4-0	pks	24	K.S	-22.4
5-A	np	87	N	86.0
6-A	npk	133	N.K	7.0
7-A	nps	126	N.S	-0.3
4-A	npks	106	N.K.S	-10.7
8-0	(1)	13	Mean	47.8
5-0	p	19	P	10.2
8-A	n	72	N	63.8
5-A	np	87	N.P	4.8
			Mean †	87.9
			N	92.1

* Mean and effects of N, K, and S are all in presence of phosphate.

† Mean and effect of N determined from all plots.

1930. Comparison of the results in Table II with the yields of the whole plots at harvest suggests that the estimates of dry-matter production from the samples for plots 5 and 11 were too low and those for plot 7 too high; the small reduction caused by the *pks* dressing applied alone, and the larger response to *s* than to *k*, shown in Table II, were reversed in the harvest yields.

Dry-matter production in the barley experiment was greatly increased by nitrogenous fertilizer. Phosphate and the mixture of potassium, sodium, and magnesium salts also caused increases, which were greater in the presence of nitrogen than in its absence. The P.KS interaction was negligibly small. Farm-yard manure produced a very large increase, greater than that of the complete fertilizer dressing (*npks*). The harvest yields agreed closely with these results, except that they showed a relatively smaller phosphate effect. Evidently the response to phosphate decreased in the interval of seven weeks between the last sampling on July 7 and harvest. This may be explained by the effect of phosphate in hastening the ripening of the crop, which is very obvious in this experiment.

In the mangold experiment the increase of dry-matter production caused by nitrogenous fertilizer was very great; the mean of all plots receiving nitrogen was about three times that of the plots without nitrogen. In the first factorial group of treatments farm-yard manure produced nearly as great an increase as nitrogen. The effect of phosphate and potash applied together (PK) was smaller but still very considerable. In its interactions farm-yard manure behaved more like a fertilizer supplying phosphate and potash than as a nitrogenous fertilizer, for there were positive N.PK and D.N interactions, of similar magnitude, but the D.PK interaction was very small.

Potash and the mixture of sodium and magnesium salts, tested in the second factorial group, had almost equal effects. Each caused a large increase of dry-matter production when applied in the absence of the other, but a reduction if the other was present. The effect of the combined dressing (*ks*) was much less than those of either component. This strong negative K.S interaction was confirmed in the yields obtained from whole plots at harvest. It was more marked in 1939 than in other seasons. All these effects, and especially that of potash, were increased by the presence of nitrogen.

Phosphate also increased dry-matter production, but its effect, estimated from the third factorial group, was small compared with those of the other fertilizers.

2. Net assimilation rate

The magnitudes of the effects on NAR (Table III) varied somewhat irregularly in successive sampling intervals, presumably because of sampling errors, but no steady drifts with time were detectable, and it is unnecessary to present the results for each sampling interval. For wheat, only means for the whole period of observation are given. For barley and mangolds the effects in the period when leaf area was increasing appeared to differ from those in

TABLE III

Net Assimilation Rate (gm. per sq. metre per week)

WHEAT. Means for period, April 25-June 20.

Treatment.	After crop.	After fallow.	Mean.
d	31	28	29
(1)	30	44	37
pks	22	26	24
npks	33	34	33
n	35	36	36
np	22	29	26
npk	21	27	24
Mean	28	32	30

BARLEY.

Means for period:

Means for period:

Treat- ment.	May 16- June 14.	June 14- July 7.	May 16- July 7.	Effect.	May 16- June 14.	June 14- July 7.	May 16- July 7.
(1)	25	53	37	Mean	31.8	52.4	40.6
p	26	58	40	P	5.4	-2.0	2.2
ks	28	58	41	KS	2.9	-4.3	-0.2
pks	26	45	34	P.KS	0.8	4.8	2.5
n	31	62	44	N	10.6	-2.1	5.1
np	39	45	42	N.P	6.2	2.6	4.7
nks	32	40	35	N.KS	1.4	-0.3	0.7
npks	47	58	52	N.P.KS	2.3	13.6	7.2
(1)	29	37	32				
d	34	46	39	D	5.3	8.6	6.8

MANGOLDS.

Means for period:

Means for period:

Treat- ment.	July 14- Sept. 6.	Sept. 6- Oct. 18.	July 14- Oct. 18.	Effect.	July 14- Sept. 6.	Sept. 6- Oct. 18.	July 14- Oct. 18.
(1)	33	23	29	Mean	55.9	30.2	44.9
pk	51	32	43	PK	4.8	4.4	4.6
d	62	7	38	D	13.7	-5.9	5.3
dpk	64	30	49	D.PK	-6.6	6.3	-1.1
n	53	45	50	N	6.7	14.3	10.0
npk	58	32	47	N.PK	-5.2	-11.9	-8.1
dn	66	37	53	D.N	-6.7	3.0	-2.5
dnpk	60	35	49	D.N.PK	1.5	-0.5	0.6
p	43	4	26	Mean*	55.4	21.6	40.9
pk	51	32	43	K	2.1	-1.0	0.8
ps	43	35	40	S	2.9	-3.7	0.1
pks	53	1	31	K.S	0.6	-18.4	-7.5
np	64	26	47	N	15.4	7.2	11.9
npk	58	32	47	N.K	-7.0	1.7	-3.5
nps	68	24	49	N.S	1.6	-3.8	-0.7
npks	63	19	44	N.K.S.	-0.2	12.9	5.4
(1)	33	23	29	Mean	48.3	24.4	38.0
p	43	4	26	P	9.9	-19.3	-2.6
n	53	45	50	N	20.5	22.3	21.3
np	64	26	47	N.P	0.4	-0.2	0.2
				Mean†	55.8	25.0	42.6
				N	11.7	12.4	12.0

* Mean and effects of N, K, and S are all in presence of phosphate.

† Mean and effect of N determined from all plots.

the subsequent period. To show this the whole period of observation has been divided into two parts at about the time of maximum leaf area, and separate means are given for the earlier and later periods of growths as well as for the whole period. Such a division would be desirable even if the effects were independent of time, for the errors of estimation of N A R increase as growth progresses. Consequently the observations for the earlier period provide more sensitive tests of treatment effects than the later observations.

Wheat. Considering first the means of both sections, after crop and after fallow, it is apparent that nitrogen increased N A R in the presence of the mixture of mineral fertilizers (*pks*), but had little effect in its absence. The mineral fertilizers reduced N A R, the reduction being greater where no nitrogen was given. Of the separate components of the mineral mixture, phosphate and potash caused a reduction, while the sodium and magnesium salts (*s*) increased N A R. Farm-yard manure reduced N A R, behaving in this respect like the complete fertilizer-dressing (*npk*).

The mean N A R for the section after fallow was greater than that for the section after crop. This is in accordance with the effect of nitrogenous fertilizer, for fallow causes an accumulation of nitrate in the soil. However, the effect of fallow differed from that of nitrogenous fertilizer in being smaller where the mixture of mineral fertilizers (*pks*) were applied than where no fertilizer was given. Another anomalous result is that fallow caused a small depression of N A R on the plot which received farm-yard manure.

So far as it is possible to generalize from these not completely concordant results, it appears that addition of nitrogenous fertilizer increased N A R, especially on the section after crop where the supply of nitrogen from the soil was low. Phosphate and potash, on the other hand, had an adverse effect on N A R, which more than counterbalanced the beneficial effect of nitrogen, when nitrogen, phosphate, and potash were given together, either as a fertilizer mixture or in the form of farm-yard manure. The mixture of sodium and magnesium salts, unlike potash, apparently caused an increase in N A R.

Barley. In the first period, when leaf area was increasing, the main effects of the three factors, N, P, and KS, and all their interactions, were positive; all the factors increased N A R, and the effect of each was enhanced by the presence of the others. Nitrogen had the greatest effect and increased N A R in all conditions. The phosphate response was negligible in the absence of nitrogen, but large in its presence. The effect of the mixture of potassium, sodium, and magnesium salts (KS) was much smaller than that of either nitrogen or phosphate. When all fertilizers were applied together, N A R was nearly twice as great as when no fertilizer was applied.

The effects in the second period, when leaf area was declining, were much more complicated. All the main effects became negative, while the interactions remained positive except for N.KS, which was negligibly small, and the second-order interaction N.P.KS was very large. The significance of this is most easily understood by considering the values of N A R for the different treatment combinations. Each of the factors applied alone, and all three

applied together, caused an increase in N A R, but when they were applied together in pairs, that is, in conditions where one factor was absent but the other two present, N A R was depressed below the value found when no fertilizer was applied. The explanation may be that application of two of the nutrient factors, when the third was omitted, produced a lack of balance in the nutrient content of the leaf, which caused a more rapid decline in the efficiency of the photosynthetic system during the phase of senescence.

The means for the whole period of observation, May 16–July 7, are of less interest in view of the great differences between the effects in the early and late stages. They show that on the average of all times and treatments, the nitrogenous fertilizer caused nearly twice as great an increase of N A R as the phosphate fertilizer, while the average response to the mixture of potassium, sodium, and magnesium salts was negligibly small.

Farm-yard manure increased N A R in both periods; its effect was smaller than that of the complete fertilizer-dressing (*npks*).

The values of N A R for the two unmanured plots differed considerably, especially in the later period, May 16–July 7. This may have been due to soil-heterogeneity, as the two plots are widely separated, being situated at opposite sides of the field.

Mangolds. In the period July 14–September 6 all the treatments of the first factorial group, farm-yard manure, nitrogen, and the combined phosphate and potash dressing (*pk*), had positive effects on N A R. The response to farm-yard manure was much the greatest. All the first-order interactions were negative and the second-order interaction was small in comparison with the other effects. In presence of farm-yard manure, the addition of fertilizers made little difference, although in its absence they produced large increases. Farm-yard manure supplies nitrogen, phosphate, and potash, and the negative D.PK and D.N interactions may imply that the responses to *n* and *pk* decline as the level of supply increases. Alternatively, the D.PK, D.N, and N.PK interactions may all be interpreted as interactions between nitrogen and potash, for the N.K interaction in the second factorial group of treatments was negative in this period, while the N.P interaction in the third group was negligibly small.

In the period September 6–October 18 the large increase of N A R produced earlier by farm-yard manure disappeared and was replaced by a depression. This is difficult to understand, as the effect of nitrogen was greater in the second period than in the first, and the PK effect was almost unchanged. The negative response to farm-yard manure was largely determined by the very low value of N A R for the plot receiving farm-yard manure alone (*d*), but there was also a small depression by farm-yard manure in the presence of the fertilizer treatments. The negative N.PK interaction was larger in the second period; phosphate and potash increased N A R only in the absence of nitrogen, and caused a reduction in its presence, but the nitrogen response was consistently positive, though smaller where phosphate and potash were also applied.

The means for the whole period of observation show a large nitrogen response, smaller effects of farm-yard manure and PK, and a large negative N.PK interaction; the other interactions were relatively small.

The nitrogen responses in the second and third factorial groups of treatments were even larger than in the first group. The results for the second group differ from those of the first in that the effect of nitrogen apparently fell in the later stages of growth. The mean response to nitrogen determined over all plots was almost identical in the early and late periods.

The effects of potassium, sodium, and magnesium salts estimated from the second group of treatments were much smaller than the effect of nitrogen. In the first period potash increased N A R in the absence of nitrogen, but decreased it where nitrogen was applied. The mean effect of the sodium and magnesium salts was similar in magnitude to that of potash, but the interaction with nitrogen was small and of the opposite sign; the response to sodium and magnesium was slightly greater in the presence of nitrogen than in its absence. It is difficult to draw any conclusions from the results for the second period. Two of the plots (treatments *p* and *pks*) gave very low values of N A R compared with the others, possibly because of sampling errors. The largest treatment effects were the K.S. and N.KS interactions. Both potash and the mixture of sodium and magnesium salts appeared to reduce N A R, especially when applied in combination. The means for the whole period of observation give no convincing evidence that N A R was affected by either potassium or sodium and magnesium salts, though there is an indication of a response to potash in the absence of nitrogen.

In the third factorial group of treatments, phosphate increased N A R in the first period but decreased it in the second; on the average of both periods the phosphate effect was small and negative.

Reviewing all three experiments, the most clear-cut effect is that of nitrogenous fertilizer; this consistently increased the N A R of all the crops, and the increase persisted throughout the whole period of the observations.

The effects of the other nutrients were more erratic. In the early stages of growth of barley and mangolds there were well-defined positive effects of phosphate. The same is true of the dressings of potassium, sodium, and magnesium salts. But where potassium was tested separately on mangolds its effect in the first period varied with the level of nitrogen supply; it reduced the N A R of mangolds which received nitrogenous fertilizer, and a similar reduction in the presence of nitrogen was found in the wheat experiment. The mixture of sodium and magnesium salts did not show this strong negative interaction with nitrogen. On the contrary, it increased the N A R of mangolds whether nitrogen was given or not; it also increased the N A R of wheat.

In the later period when leaf area was decreasing, the interactions between the effects of the nutrient factors became large compared with the main effects. This may have been partly the result of an increase in the magnitude of sampling errors in the later stages of growth. In the barley experiment the interactions may reflect variations in the rate of senescence of the leaves, but

it is doubtful whether senescence plays any considerable part in determining the results for mangolds. In contrast with the cereals, mangolds do not die at the end of their first year of growth, and it is not certain whether the fraction of the total leaf area undergoing senescence increases towards the end of the growth period. The average effects of phosphate, potash, and the mixture of sodium and magnesium salts were negative in this period, the only exception being that the PK effect in the first factorial group of treatments of mangolds was positive.

Farm-yard manure increased the N A R of barley in both periods, and of mangolds in the first period only; it reduced the N A R of wheat, and of mangolds in the second period. On the whole its effect was similar to that of a fertilizer mixture supplying nitrogen, phosphate, and potash, but the results for mangolds in the later period were anomalous in this respect.

3. *Leaf area*

The average effects of the nutrient factors on leaf area per metre of drill-row or per plant are presented in Tables IV to VI. These give means for each plot and estimates of the main effects and interactions derived from the factorial groups of treatments, averaged over all dates of observation. The tables also show the average effects on the number of leaves per plant and on the mean area per leaf in mangolds, and on shoot number per metre and mean leaf area per shoot in cereals. These results provide a basis for distinguishing between effects on total leaf area produced by variation in meristematic activity and effects produced by changes in the growth of individual organs. The measure of meristematic activity is not the same for the cereals as for mangolds, but it is probably safe to interpret shoot number per metre of the cereals as a measure of leaf number, and leaf area per shoot as a measure of leaf size, for differences in leaf number per shoot at any time of observation were probably small, though this would obviously not be true of comparisons made between different dates of observation.

The changes with time in these attributes and in the effects on them of variation in nutrient supply are shown in Figs. 1 to 3, where the mean of all plots and the main effects of each factor are plotted for each date of observation.

Wheat (Table IV, Fig. 1). The mean leaf area per metre (Fig. 1) continued to rise throughout the period of observation. The number of shoots per metre fell rapidly from its maximum, which occurred before the observations began, to a constant level in June, while leaf area per shoot was increasing to a maximum at, or near to, the end of the period.

Farm-yard manure and all the fertilizer treatments increased leaf area per metre. The effect of farm-yard manure was the greatest, and it increased steadily throughout the period. The response to nitrogen varied little with time. The complete mineral fertilizer-dressing (*pks*) caused a large increase at the first observation in April, but its effect subsequently fell to a low value at the end of the period. Although the effects of the individual components

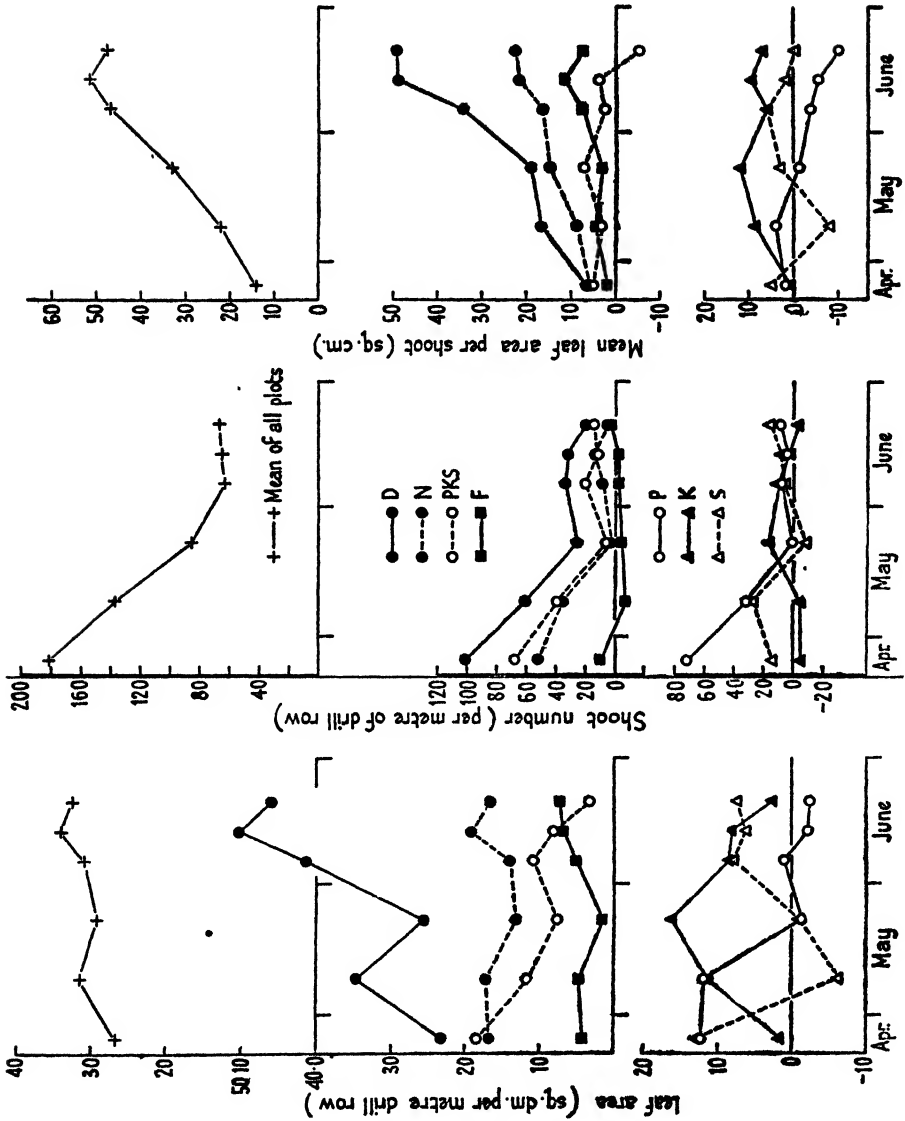


FIG. 1. *Wheat, Broadbalk.* Changes with time in leaf area, sq. dm., per metre of drill-row, shoot number per metre of drill-row, and mean leaf area per shoot, sq. cm. The top row of graphs shows the mean values of these attributes taken over all plots at each time of observation; the middle row shows the average responses to fallow (F), farm-yard manure (D), nitrogenous fertilizer (N), and a complete dressing of mineral fertilizers (PKS); the bottom row shows the responses to successive additions of phosphate (P), potash (K), and the mixture of sodium and magnesium salts (S), all in presence of nitrogen.

of the mixture varied erratically with time, presumably because of sampling errors, it is clear that the fall in the PKS effect was mainly attributable to the phosphate component. The average response to potash was greater than the response to the mixture of sodium and magnesium salts.

TABLE IV. *Wheat: Leaf Area (sq. dm. per metre of drill-row), Shoot Number (per metre of drill-row), and Leaf Area (sq. cm. per shoot). Mean of all Dates of Observation*

Treatment.	Leaf area per metre.			Shoot number per metre.			Leaf area per shoot.		
	C.	F.	Mean.	C.	F.	Mean.	C.	F.	Mean.
d	40	64	52	106	129	118	47	59	53
(1)	13	17	15	78	67	72	19	29	24
pks	14	25	19	84	100	92	19	28	24
npks	36	46	41	116	120	118	38	45	42
n	31	20	25	99	70	85	37	35	36
np	31	26	28	107	103	105	33	33	33
npk	37	36	37	111	105	108	38	43	41
Mean	29	33	31	100	99	100	33	39	36

C = after crop, F = after fallow.

Farm-yard manure and nitrogenous fertilizer increased both shoot number and leaf area per shoot throughout. The *pks* treatment caused an increase in shoot number at all times; it also increased leaf area per shoot at the earlier observations, but its effect fell to zero at the end of the period. Phosphate tended to increase shoot number, but caused a decrease in leaf area per shoot in June. Potash had little effect on shoot number, but consistently increased leaf area per shoot.

On the average of all plots, fallow increased both leaf area per metre and per shoot, but caused a small depression of shoot number per metre. The means plotted in Fig. 1 do not adequately describe the effects of fallow, which, as Table IV shows, varied widely between plots. Where farm-yard manure or the complete mixture of mineral fertilizers (treatments *d*, *pks*, and *npks*) was applied, leaf area per metre and per shoot, and shoot number per metre, were all greater after fallow than after crop, and the responses to fallowing varied with time in a similar way to the nitrogen responses. On the other plots fallowing caused a reduction in shoot number per metre. Where both potash and the mixture of sodium and magnesium salts were omitted (treatments *n* and *p*), this led to a reduction of leaf area per metre, as leaf area per shoot was almost unaffected, but on the remaining plots (treatments (1) and *npk*) the reduction in shoot number was offset by an increase in leaf area per shoot, so that leaf area per metre was scarcely changed by fallowing.

✓ It is known that fallowing leads to an accumulation of nitrate in the soil, and the effects of fallow in the presence of a complete supply of mineral nutrients, applied in the form of fertilizers or farm-yard manure, were similar to those produced by nitrogenous fertilizer. If the effect of fallow on the other plots is to be attributed to increased nitrogen supply, it is necessary to

assume that excess nitrogen in conditions of deficiency of other nutrients had harmful effects on meristematic activity, causing a reduction of tillering. No independent evidence to support this assumption can be quoted. In barley Mathur (see Gregory, 1937) found no reduction in shoot number with increasing supply of nitrogen over a wide range, at low levels of potassium supply.

Barley (Table V, Fig. 2). The leaf area per metre of drill-row and per shoot, averaged over all plots (Fig. 2), rose to a maximum in late June and subsequently fell. Shoot number per metre reached its peak at the end of May, then decreased throughout June, but showed little change in the period between the last two dates of observation.

TABLE V. *Barley: Leaf Area (sq. dm. per metre of drill-row), Shoot Number (per metre of drill-row), and Leaf Area (sq. cm. per shoot). Mean of all Dates of Observation*

Treat- ment.	Leaf area per metre.	Shoot number per metre.	Leaf area per shoot.	Effect.	Leaf area per metre.	Shoot number per metre.	Leaf area per shoot.
(1)	11	48	24	Mean	18.7	64.4	29.2
p	13	54	24	P	4.3	13.3	0.9
ks	12	53	23	KS	1.8	3.6	1.6
pks	14	56	26	P.KS	-2.0	-6.9	-0.0
n	18	58	32	N	12.5	23.6	9.9
np	29	91	33	N.P.	2.5	8.5	-0.7
nks	25	73	36	N.KS	1.0	0.2	1.3
npks	28	83	35	N.P.KS	-2.1	5.0	-1.1
(1)	12	44	26				
d	36	86	45	D	24.4	41.7	18.5

Farm-yard manure and nitrogenous fertilizer both caused large increases in leaf area per metre, and their effects showed a time-trend similar to that of the mean of all plots. Each of them increased both shoot number per metre and leaf area per shoot, at all times of observation.

Phosphate also increased leaf area per metre, but only during the early part of the period; it ceased to have any effect after the time when mean leaf area per metre reached its maximum. It increased shoot number per metre throughout, and in May and early June it also increased leaf area per shoot, but during the phase of decreasing leaf area it tended to depress leaf area per shoot.

The changes with time in the effects of the mixture of potassium, sodium, and magnesium salts (*ks*) were the inverse of those found for phosphate. The effect of *ks* on leaf area per metre increased with time and reached its highest values in the period when mean leaf area per metre was decreasing. Before this the effect was small, for although *ks* caused an increase in shoot number per metre, it also slightly reduced mean leaf area per shoot. In the period when mean leaf area per metre was decreasing, *ks* had no effect on shoot number per metre, but caused an increase in leaf area per shoot. The

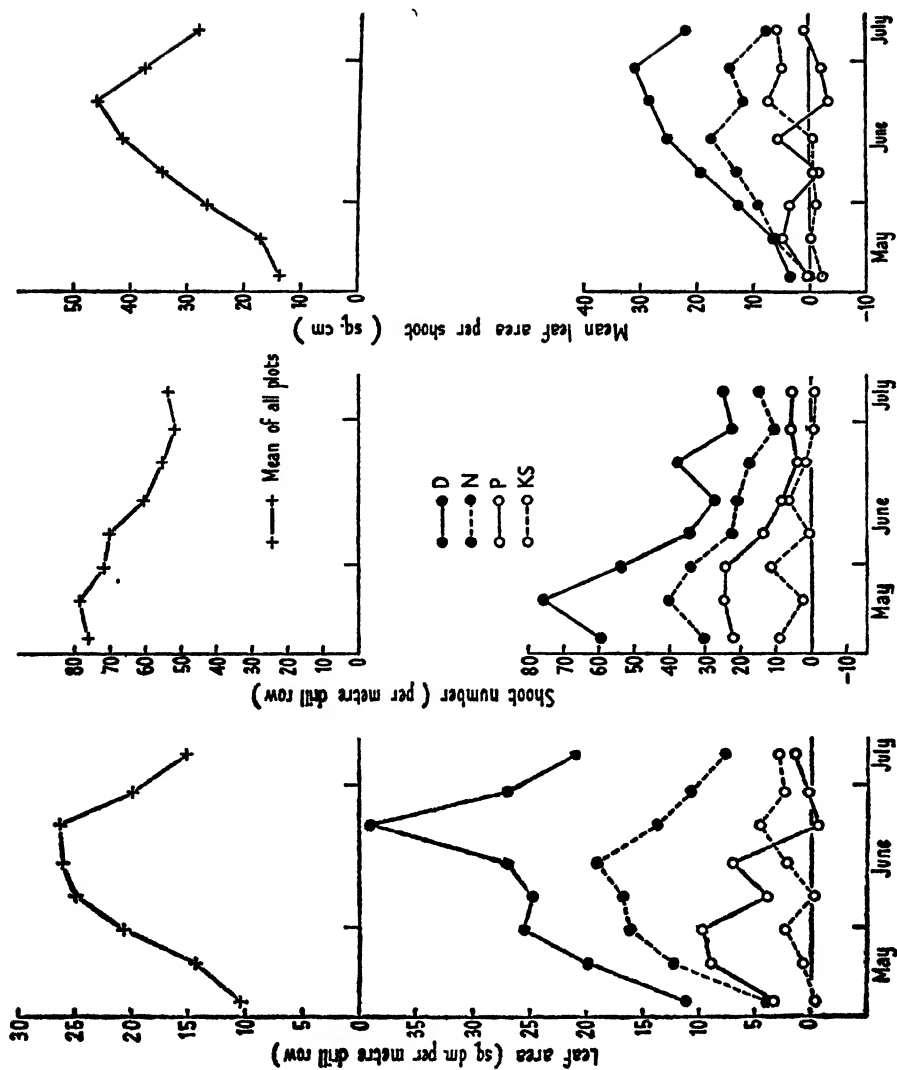


FIG. 2. *Barley, Hoosfield.* Changes with time in leaf area, sq. dm. per metre of drill-row, shoot number per metre of drill-row, and area (sq. cm.) per shoot. The upper row of graphs shows the mean values of these attributes taken over all plots at each time of observation; the lower row shows the average responses to farm yard manure (D), nitrogen (N), phosphate (P), and the mixture of potassium, sodium, and magnesium salts (KS).

explanation of this contrast between the phosphate and KS effects is presumably that the mixture of potassium, sodium, and magnesium salts delayed the senescence and death of the leaves while phosphate tended to hasten senescence.

The effects of both phosphate and *ks* on leaf area per metre were greater in the presence of nitrogen than in its absence, but the P.KS interaction was negative.

Mangolds (Table VI, Fig. 3). Mean leaf area per plant, mean area per leaf, and the mean number of leaves per plant (Fig. 3) all increased steadily during July and August. Subsequently the mean area per leaf declined, but as

TABLE VI. *Mangolds: Leaf Area (sq. dm. per plant), Leaf Number (per plant), and Mean Leaf Area (sq. cm.). Mean of all Dates of Observation*

Treat- ment.	Leaf area per plant.	Leaf number per plant.*	Mean area per leaf.	Effect.	Leaf area per plant.	Leaf number per plant.†	Mean area per leaf.
(1)	4.0	12.0	30	Mean	18.6	19.5	76.8
pk	5.2	13.6	36	PK	7.0	2.5	22.0
d	15.5	19.1 (0.4)	71	D	15.7	6.9	46.0
dpk	18.3	20.1 (0.7)	80	D.PK	1.3	0.3	-0.1
n	11.7	17.7	55	N	15.7	5.9	44.9
npk	21.9	19.8 (0.8)	94	N.PK	5.0	1.1	14.9
dn	29.0	22.9 (0.6)	107	D.N	3.4	-0.4	3.4
dnpk	43.0	26.1 (1.7)	141	D.N.PK	0.6	0.5	-2.0
p	5.7	14.2	37	Mean ‡	12.9	17.1	63.1
pk	5.2	13.6	36	K	0.5	-0.1	2.2
ps	7.4	15.0	46	S	1.2	0.5	6.3
pks	6.2	14.6	40	K.S	-2.1	-0.5	-7.5
np	16.4	19.0 (0.1)	73	N	13.6	5.5	47.1
npk	21.9	19.8 (0.8)	94	N.K	1.3	0.4	5.9
nps	21.2	19.9 (0.4)	92	N.S	-0.1	-0.4	0.1
npks	19.2	18.9 (0.6)	87	N.K.S	-1.7	-0.6	-5.4
(1)	4.0	12.0	30	Mean	9.4	15.7	48.9
p	5.7	14.2	37	P	3.2	1.7	12.4
n	11.7	17.7	55	N	9.2	5.2	30.2
np	16.3	19.0 (0.1)	73	N.P	1.5	-0.5	5.6
				Mean §	16.0	18.5	70.7
				N	14.3	5.5	44.3

* Leaves produced from the apical bud. Number of leaves produced from axillary buds given in brackets. † All leaves included. ‡ Mean and effects of N, K, and S are all in presence of phosphate. § Mean and effect of N determined from all plots.

the number of leaves per plant continued to increase, though more slowly, there was only a slight decrease in leaf area per plant in September and October.

Farm-yard manure and nitrogenous fertilizer caused large increases in leaf area per plant. The effects of both increased with time and persisted at a high level until the end of the period. Farm-yard manure produced a greater response than nitrogen throughout July and August, but later they were nearly equally effective. Phosphate and potash applied together, in the first factorial group of treatments, at first caused as large an increase in leaf area per

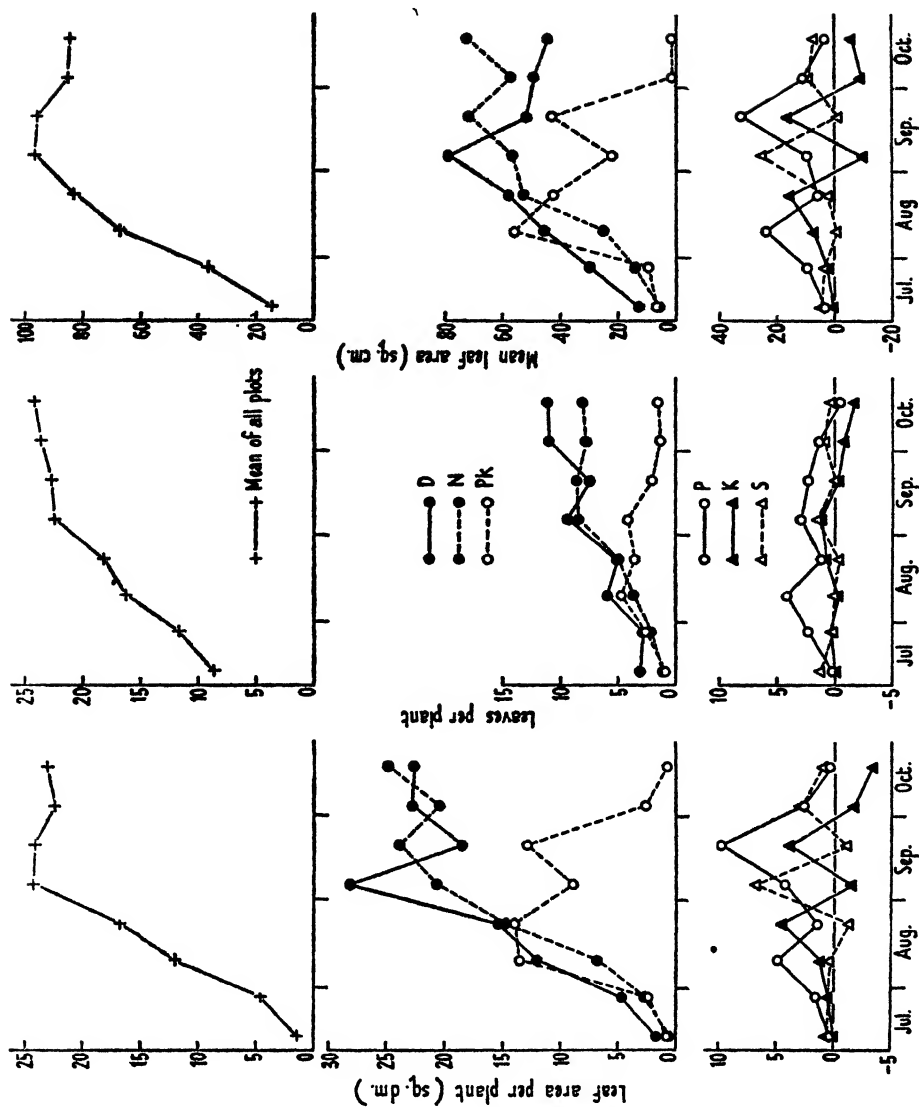


FIG. 3. *Mangolds, Barnfield.* Changes with time in leaf area per plant, sq. dm., leaf number per plant (including leaves produced from both apical and axillary buds), and mean area per leaf, sq. cm. The top row of graphs shows the mean values of these attributes taken over all plots at each time of observation; the middle row shows the average responses to nitrogen (N) determined from all plots, and to farm-yard manure (D), and to phosphate and potash applied together (PK), in the first factorial group of treatments; the bottom row shows the average responses to potash (K) and the mixture of sodium and magnesium salts (S), in the second factorial group, and to phosphate (P) in the third factorial group.

plant as farm-yard manure and nitrogen, but during September the response began to decrease and fell almost to zero in October. Table VI shows that the effect of each of these factors was increased by the presence of the others.

All three factors increased the number of leaves per plant. Farm-yard manure had a slightly greater effect than nitrogenous fertilizer; the effects of both increased steadily during July and August, subsequently remaining fairly constant. Phosphate and potash (*pk*) also increased leaf number per plant throughout, but the effect was maximal in early August and decreased later.

Table VI shows that leaf production from axillary buds made only a small contribution to the total number of leaves per plant, and occurred only in plants which received either farm-yard manure or both nitrogen and phosphate; apical dominance was complete if either nitrogen or phosphate were lacking. Potash and the mixture of sodium and magnesium salts, applied in the presence of nitrogen and potash, both increased the number of leaves produced from lateral buds.

In 1946 counts of leaf number were made on samples of mangolds growing on the same plots as were used in 1939. Every fifth leaf was marked so that the total number of leaves produced and the number dead at any time could be determined. Counts were made at weekly intervals from July 5 to August 23. The mean number of leaves per plant, produced from the apical bud, present on August 23, the mean rates of production and death of leaves over the period, and the effects of farm-yard manure, nitrogenous fertilizer, and the *pk* dressing were as follows:

	Number of leaves (per plant) 23 August.	Rate of production (per plant per week).	Death-rate (per plant per week).
Mean	20.7	2.53	1.05
Effect of { PK	-0.5	-0.21	-0.13
{ D	8.0	0.81	0.18
{ N	5.3	0.77	0.26

These results show that farm-yard manure and nitrogenous fertilizer increased leaf number per plant by increasing the rate of leaf production, and not by prolonging the life of the leaves. Both treatments increased the death-rate as well as the rate of production. The *pk* dressing had little effect on leaf number, apparently causing a small depression instead of the increase found in 1939.

Nitrogenous fertilizer, farm-yard manure, and the *pk* dressing all caused large increases in mean area per leaf. The nitrogen response increased rapidly during July and August, and then more slowly. The PK response reached a maximum in early August, and later decreased almost to zero in October. The time trend of the farm-yard manure response was of an intermediate type; it increased during July and August and throughout this period was greater than the nitrogen response, but from the beginning of September it showed a fall, like that in the PK response, though it was still large at the last observation in October. This is consistent with the fact that farm-yard manure supplies phosphate and potash as well as nitrogen.

Potash and the mixture of sodium and magnesium salts, tested in the second factorial group of treatments, both increased leaf area per plant in the presence of nitrogen, but had little effect in its absence. The K.S interaction was negative and larger than either of the main effects; the *ks* dressing produced a smaller increase than either *k* or *s* applied alone. The effect on leaf area was brought about by an increase in mean area per leaf, for leaf number was scarcely affected. Phosphate, tested in the third factorial group, increased leaf area per plant by increasing both leaf number and leaf size. The variation with time in the P, K, and S responses were irregular, and little can be said about them, except that all the effects were greater in the middle of the period of observation than at the beginning or the end. There is an indication that potash caused a small depression of leaf area per plant, leaf number, and leaf size in October.

It is difficult to draw general conclusions about the effects of the different nutrients on leaf area because the conditions in which the effects were measured were not the same in all three experiments. Both the treatment combinations tested and the rates of application varied. However, some measure of uniformity in the effects is obvious. In all three experiments nitrogenous fertilizer produced increases in leaf area which persisted throughout the period of observation, in contrast with the more transitory increases produced by phosphate and potash. In the two cereal experiments phosphate increased leaf area per metre mainly by increasing tillering, and its effect was greatest near to the time of maximum shoot number. Subsequently it declined during the phase when tillers were dying off, so that from the middle of June onwards it was no longer apparent. The effects of potash, and the mixture of sodium and magnesium salts, on the other hand, were small during the tillering phase but increased during the phase of shoot extension in May and June. This time difference is important from the point of view of dry-matter production, because N A R was near to the maximum of its seasonal trend at the time when the potassium and sodium effects were manifested (Watson, 1947), and was much greater than in April and May when phosphate had its greatest effect. A similar difference between phosphate on the one hand and potassium and sodium salts on the other, in their effects on the activity of the apical meristem, was shown in the mangold experiment; phosphate increased the number of leaves per plant, while potassium and sodium did not. But in this crop phosphate also affected leaf size. In all three experiments farm-yard manure appeared to act principally as a source of nitrogen, producing effects on leaf area similar to, but greater than, those of nitrogenous fertilizer, but in the mangold experiment there was an indication that part of its effect was attributable to the phosphate and potash which it supplied.

4. The relative magnitudes of the effects on N A R and on leaf area

The relative importance of the contributions made by changes in N A R and leaf area to determine the effects of the treatments on dry-matter production can be assessed by inspection of Table VII, where each of the main effects,

averaged over all times of observation, given in Tables II and VI, is expressed as per cent. of the mean of all plots from which the effect was computed.

It is obvious from Table VII that the average effects on leaf area per metre or per plant were much larger than those on N A R. In all three experiments N A R was markedly increased by nitrogenous fertilizer, but the per cent. increase of leaf area which it caused was 3 to 5 times as great as that of N A R. The other factors were more variable in their effects on N A R, but when they increased it they had an even greater effect on leaf area; when they had no effect on N A R or caused a reduction they still increased leaf area.

TABLE VII

Average Effects of Manurial Treatments on Dry-matter Accumulation, on N A R, and on Leaf Area (results as per cent. of the mean)

Effect.	WHEAT.						
	Fallow.	D.	N.	PKS.	P.	K.	S.
Dry-weight increase, gm. per metre	23	90	70	26	-17	22	40
N A R.	13	-24	12	-25	-32	-8	32
Leaf area, sq. dm. per metre	16	111	64	40	12	25	12

Effect.	BARLEY.			
	D.	N.	P.	KS.
Dry-weight increase, gm. per metre	112	72	20	11
N A R.	19	13	5	0
Leaf area, sq. dm. per metre	102	67	23	10

Effect.	MANGOLDS.					
	D.	N.	PK.	P.	K.	S.
Dry-weight increase, gm. per plant	81	105	42	21	8	9
N A R.	12	28	10	-7	2	0
Leaf area, sq. dm. per plant	84	89	38	34	4	9

Effects were computed from the following plots: *Wheat*. Fallow: all plots. D: plots 2 and 3. N and PKS: plots 3, 5, 7, and 10. P: plots 10 and 11. K: plots 11 and 13. S: plots 7 and 13. *Barley*. D: plots 7-1 and 6-2. N, P, and KS: plots 1, 2, 3, and 4 of Series O and A. *Mangolds*. N: all plots. D and PK: plots 1, 2, 6, and 8 of Series O and A. P: plots 5 and 8 of series O and A. K and S: plots 4, 5, 6, and 7 of series O and A. The effects were expressed as per cent. of the mean for all plots involved in their computation.

Two exceptions to this general statement are apparent in Table VII; in the wheat experiment fallowing caused nearly as great an increase in N A R as in leaf area, while the mixture of sodium and magnesium salts (s) was more effective in increasing N A R than leaf area. The anomalous result of fallowing was restricted to the plots where one or more of the mineral nutrients were deficient (p. 389). Here fallow caused an increase in N A R, while leaf area was unaffected or reduced. On the plots which received farm-yard manure or complete mineral fertilizer, fallow behaved like nitrogenous fertilizer, increasing dry-matter production mainly through an effect on leaf area.

Other exceptions can be found if account is taken of the interactions between

treatments instead of considering only the main effects. In the barley experiment each of the factors *n*, *p*, and *ks*, in the presence of both of the others, increased N A R, but decreased it when only one of the others was present. The per cent. responses of dry-weight increase, N A R, and leaf area to each factor, when both of the others were applied, were as follows:

Effect.	N.	P.	KS.
Dry-weight increase .	84	25	15
N A R.	42	39	21
Leaf area per metre .	67	11	-4

In these conditions the effect of *ks* on dry-matter yield was accounted for entirely, and that of phosphate mainly, by increase of N A R. Nitrogenous fertilizer conformed to the general rule, having a greater effect on leaf area than on N A R.

Because of the negative K.S interactions in the mangold experiment (Tables II, III, and VI), the main effects of *k* and *s* shown in Table VII are not very informative, and conceal the fact that there were large responses to both factors. To show the nature of these responses it is necessary to consider the effects of *k* and *s* applied separately and in combination. Table II shows that the effects of these treatments on N A R were dependent on whether or not nitrogenous fertilizer was supplied. The effects of the *k*, *s*, and *ks* dressings on dry-weight increase, on N A R, and on leaf area per plant, each expressed as per cent. of the mean of all plots involved in its estimation, are set out below:

	<i>n</i> absent.			<i>n</i> present.			Mean of <i>n</i> absent and present.		
	<i>k</i> .	<i>s</i> .	<i>ks</i> .	<i>k</i> .	<i>s</i> .	<i>ks</i> .	<i>k</i> .	<i>s</i> .	<i>ks</i> .
Dry-weight increase .	42	64	23	42	37	20	42	42	20
N A R	49	42	18	-1	4	-7	20	19	2
Leaf area per plant .	-9	26	8	32	27	17	21	26	14

All these effects were estimated from plots which received phosphate. Where nitrogenous fertilizer was given, *k*, *s*, and *ks* all had negligible effects on N A R, but increased leaf area. In the absence of *n* their effects on N A R were large and exceeded those on leaf area; *k* actually caused a small depression of leaf area. This relation of the effect of *k* and *s* to nitrogen supply was the opposite of that found in the barley experiment, where the *ks* dressing in the presence of nitrogen and phosphate increased N A R and had no effect on leaf area, but decreased N A R and increased leaf area when phosphate but no nitrogen was given. On the average of plots with and without nitrogenous fertilizer, *k* and *s* had almost equal effects on N A R and leaf area, increasing both by about 20 per cent.

These results show that, with the few exceptions noted, varying the supply of nutrients affected dry-matter production mainly by causing changes in leaf area, and that variation in N A R was of secondary importance. This is perhaps more clearly demonstrated in Fig. 4, where the increase in dry-weight per plant over the period of observation is plotted against mean leaf area per

plant and mean N A R for the different plots of the mangold experiment. It is obvious from the figure that leaf area varied more widely between plots, relative to its mean value, than N A R. Dry-matter production increased almost linearly with increase in mean leaf area over the whole range. In contrast with this, dry-weight increase varied over the range from 100 to 230 gm. per plant without any considerable change in mean N A R, but the lower

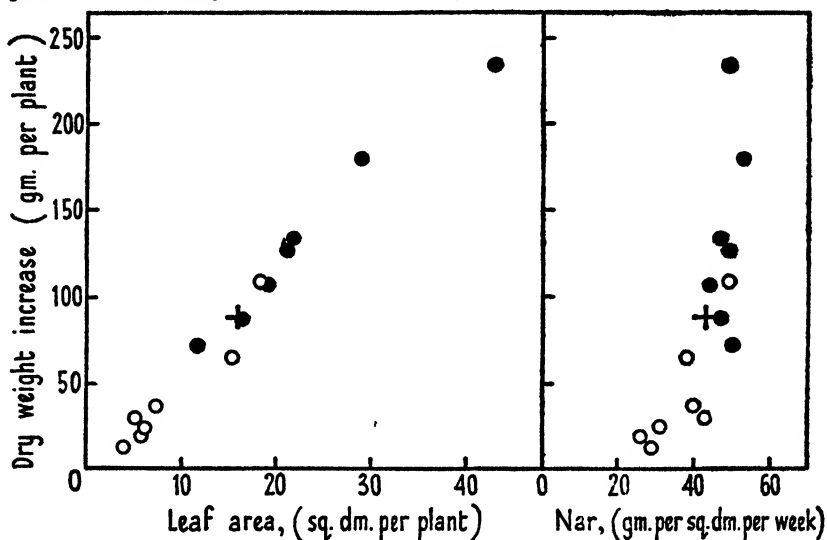


FIG. 4. The relation of dry-weight increase per plant in the experimental period to mean leaf area per plant and to mean net assimilation rate for the plots of the mangold experiment. The scales of leaf area and N A R are adjusted so that the abscissae of the mean values (represented by crosses) are approximately equal. Open circles represent plots which received no nitrogenous fertilizer (series O), and black circles plots which received sulphate of ammonia (series A).

values of dry-weight increase, for the plots which received no nitrogenous fertilizer or farm-yard manure, were associated with low values of N A R. A linear regression on mean leaf area accounted for 97 per cent. of the variance of dry-weight increase. The corresponding figure for a regression on mean N A R was only 54 per cent., and for a multiple regression on both leaf area and N A R 99 per cent. Dry-weight increase was obviously much more closely correlated with mean leaf area than with mean N A R. The wheat and barley experiments gave very similar results.

The conclusion reached in a previous paper (Watson, 1947), that variation of leaf area is a more important cause of differences in dry-matter production between species or varieties, or between years, than variation in N A R, can therefore be extended to include differences in dry-matter production induced by change of nutrient supply.

DISCUSSION

The effect of nitrogen supply on N A R in the experiments described in this paper is not in accordance with the results of Gregory (1926) and Mathur

(see Gregory and Baptiste, 1936, and Gregory, 1937). In pot-culture experiments on barley they found that up to the time of maximum leaf area NAR was independent of nitrogen supply, but in the subsequent period, when leaf area was decreasing from its maximum, Mathur showed that the NAR of nitrogen-deficient plants was less than that of fully manured plants. Direct measurements of the CO_2 assimilated by successive leaves (Gregory and Richards, 1929; Chinoy, see Gregory and Baptiste, loc. cit.) showed no reduction of the rate of photosynthesis by nitrogen deficiency, even at very low levels of nitrogen supply. These measurements were made at the time of full emergence of the leaves, well before the onset of senescence, and Gregory (1937) considered that the results of other workers who have recorded a reduction in the rate of photosynthesis due to nitrogen deficiency are accounted for by their use of older leaves in which senescence was hastened by nitrogen deficiency. Similarly, Gregory and Baptiste attributed the increase of NAR with increased nitrogen supply observed by Mathur after the time of maximum leaf area to the presence of a larger proportion of senescent leaves in nitrogen-deficient than in fully manured plants.

This explanation involves many assumptions which need not be discussed at length here. Its value is doubtful because the meaning of senescence is not precisely defined. It supposes that the time of maximum leaf area marks the beginning of senescence of the leaves. This is obviously not strictly true; in barley, for example, the first-formed leaves have died long before leaf area is maximal. Nevertheless, it is reasonable to assume that so long as the leaf area of a plant continues to increase, only a small part of it is in a senescent condition, however this is defined, and consequently that up to the time of maximum leaf area the influence of senescence on the activity of the whole foliage is negligible. For a plant such as barley, in which leaf production is terminated by the formation of an inflorescence, the fraction of the leaf area which is senescent must increase and eventually become large towards the end of the growth period, when no new leaves are emerging and existing ones are dying off in succession. Whether this increase occurs at or soon after maximum leaf area, or at a later stage, is not known. In other crops, such as the mangold in its first year of growth, which do not flower but continue vegetative growth until harvest, it is doubtful whether there is any considerable increase in the senescent fraction after the time of maximum leaf area. While it is safe to assume that effects observed before maximum leaf area are independent of senescence, it is not certain whether, or how soon, senescence becomes dominant when leaf area is declining in the later period.

The increase of NAR produced by increasing nitrogen supply in the present experiments cannot be explained as a phenomenon associated with senescence. In both wheat and barley experiments NAR was increased by nitrogen in the period before maximum leaf area. Table III shows that, in barley, the nitrogen effect did not increase subsequently; on the contrary, the average response became negative in the period after maximum leaf area, though in some conditions, especially in the presence of the complete mineral

fertilizer dressing (*pks*), increase in nitrogen supply still caused an increase of N A R. In the mangold experiment the effect of nitrogen was very uniform throughout the whole period of growth. This is shown in Fig. 5, where the mean N A R's of all plots with, and all without, nitrogenous fertilizer are

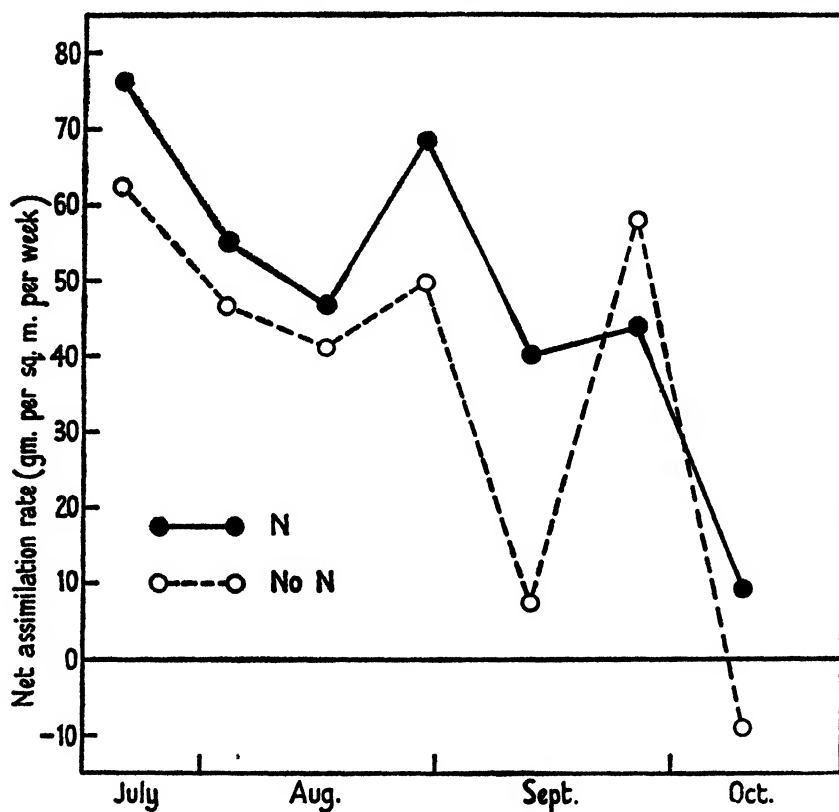


FIG. 5. The effect of nitrogenous fertilizer on the net assimilation rate of mangolds. The graphs show the mean N A R of all plots which received nitrogen (series A), and of all plots without nitrogen (series O), for each sampling interval.

plotted for each sampling interval. The response to nitrogen was already apparent in the first sampling interval. At this time there were only 9 to 12 living leaves on each plant, most of them still expanding, and though at least one leaf per plant had died before the end of the interval it is scarcely possible that the senescent fraction of the total leaf area was sufficiently large to account for the 20 per cent. increase in N A R produced by application of nitrogenous fertilizer, shown in Fig. 5. It is much more probable that the increase was caused by a difference in assimilation rate of the fully functional leaves. The nitrogen response persisted in all the later sampling intervals except the last but one. The discrepancy in this interval may be the result of sampling errors, which tend to increase greatly at the end of the growth period, when the increments in dry-weight become small compared with the total dry-weight

of the plant. Table III shows that the mean nitrogen responses in the periods before and after maximum leaf area were almost identical.

Other instances of increase in N A R produced by increased nitrogen supply in pre-senescent stages of growth are to be found in the literature. Crowther (1934) determined the N A R of cotton grown in the field in the Sudan with three rates of application of sulphate of ammonia. As statistical analysis showed no significant effect of variation in nitrogen supply on N A R, he concluded that his results were in complete agreement with those found by Gregory (1926) for barley. Inspection of Table IV of his paper suggests that, in fact, the nitrogenous fertilizer caused an increase of N A R, although the effect was not sufficiently large to attain statistical significance. The mean values of N A R for the three rates of nitrogen application were as follows (N A R was expressed on the basis of leaf dry-weight instead of leaf area; the units are not stated, but apparently are gm. per gm. leaf dry-weight per fortnight, and the values quoted are each totals for three levels of water-supply):

• Means for period.	Rate of application of sulphate of ammonia (rotls per feddan).		
	0	300	600
October 8–November 18	7.1	7.4	8.1
November 18–December 16	6.1	8.1	7.9
October 8–December 16	6.7	7.7	8.0

Leaf dry-weight was maximal on November 18 on the plots which received no sulphate of ammonia, but continued to increase until December 16 on the plots which received the largest dressing; flowering began on about November 12. Over the whole period of observation, from October 8 to December 16, nitrogen applied at the higher rate caused an apparent increase of N A R amounting to 17 per cent. of the mean value for all three treatments. The average increase of leaf dry-weight produced by this dressing in the same period, estimated from Crowther's Fig. 2, was about 50 per cent. of the mean. These results support the conclusion drawn from the present experiments, that although increased nitrogen supply increases dry-matter production mainly through an effect on leaf size, it also causes an increase of N A R, and the magnitudes of the responses are in fair agreement with those shown in Table VII.

Ballard and Petrie (1936) found that the N A R of wheat, expressed on a leaf dry-weight basis, increased with increased nitrogen supply, both before and after the time of maximum leaf dry-weight, but in an experiment on Sudan grass nitrogen treatment had no effect on N A R except in a short period after flowering. Over the whole period of the observations on wheat, including the post-flowering period when the values of N A R may have been affected by photosynthesis in the ears, the highest rate of nitrogen application caused an increase of 36 per cent. in the mean value of N A R, and about 100 per cent. in mean leaf dry-weight.

The negative interaction between nitrogenous fertilizer and farm-yard manure in the mangold experiment (Table III; first period) may indicate

that the response of N A R to nitrogen decreases as the level of nitrogen supply increases, but this is not certain, as farm-yard manure supplies potash and phosphate as well as nitrogen, and the N.PK interaction was also negative. In the wheat experiment of Ballard and Petrie there was evidence of a falling off in the response as nitrogen supply increased; the heaviest rate of application actually caused a smaller increase of N A R than the intermediate rates in the period before maximum leaf area. Crowther's experiment (p. 401) showed a similar effect. Although, then, it is possible that additional nitrogen increases N A R at low levels of nitrogen supply, but has no effect at high levels, it is unlikely that this could explain the difference between the effects of nitrogen in the present experiments and those of Gregory and Mathur. In the absence of data on nitrogen uptake per plant of the barley grown in the field and pot-culture experiments, direct comparison of the levels of nitrogen supply is not possible, but it seems probable that nitrogen deficiency at the lower levels of supply in the pots was at least as severe as in the field.

In the experiments of Verma, quoted by Gregory and Baptiste (*loc. cit.*), phosphorus deficiency had no effect on the N A R of barley in the first six weeks, but afterwards caused a depression. Williams (1936) studied the effect of three rates of application of phosphate on the growth of oats in sand-culture. N A R, expressed on a leaf dry-weight basis, was greater in the early stages of growth for the middle rate than for the lowest rate, but at about the time of maximum leaf dry-weight in one experiment, and at an earlier stage in another, the effect was reversed, and N A R was reduced by the higher phosphate application. The highest and middle rates gave almost identical values of N A R, except in one early period, when the highest rate caused a reduction. This time-drift in the response to phosphate, from positive values in the early stages of growth to negative values later, was in the opposite sense to that found by Verma, but agrees with the results of the barley and mangold experiments (Table III). At the times when N A R was increased by increased phosphorus supply in Williams's experiments, the effects of phosphate on leaf dry-weight were relatively much greater than those on N A R, and it is clear that increase of leaf size was the chief cause of the increased dry-matter yield at the higher phosphorus levels.

Williams suggested that delayed senescence of the plants with low phosphate supply might account in part for their higher N A R in the later stages of growth. This hypothesis conflicts with the statement of Gregory (1937) that phosphorus deficiency leads to more rapid senescence of leaves. Williams concluded that increased phosphorus supply hastened senescence because the dry-weights of leaves, of the whole plant, and of individual leaves reached their maxima earlier, and the ratio of the number of dead leaves to total leaf number at the time of maximum leaf weight was higher, in plants with a higher phosphorus supply. It is not clear whether the laminae of dead leaves were included in his measurements of leaf dry-weight, but if so, it is true that the increased proportion of dead leaves would cause an apparent reduction in the N A R of the plants with the higher phosphorus supply. The results

of the present experiments cannot be explained in this way, for dead leaves were excluded in estimating leaf area. Although evidence of more rapid death of leaves resulting from increased phosphorus supply was found in the later stages of the barley experiment, it is doubtful whether this accounts for the depression of N A R by phosphate. More rapid death of the leaves does not necessarily imply that the fraction of the living leaf area in a state of senescence at any time must be increased, for the death of the leaves might be hastened either by an earlier onset of senescence or by a shortening of the period of senescence of each leaf. There is no reason to suppose that the depression of N A R by increased phosphorus supply in the later period of the mangold experiment was related to senescent changes in the leaves. Petrie, Watson, and Ward (1939) found that increased phosphorus supply to tobacco plants delayed ripening, as shown by the onset of yellowing, of the group of five oldest leaves by about 7 days, but hastened ripening of the younger leaf groups by 10 days or more. Topping the plants also slightly delayed ripening. In spite of these well-established effects on the rate of senescence of the leaves, N A R was unaffected either by varying phosphate supply or by topping.

According to Gregory and Baptiste (1936), Mathur and Verma found that potassium deficiency reduced the N A R of barley throughout the life cycle, and this agreed with the results of direct measurements of the rate of photosynthesis of leaves with varied potash supply, made by Gregory and Richards (1929) and others. The results of the barley and mangold experiments described in this paper show that N A R was increased by increased supply of potassium in the early growth periods. In mangolds this increase occurred only at a low level of nitrogen supply, but in barley the positive effect of potassium on N A R was increased when additional nitrogen was supplied. In the later periods of growth, and in the wheat experiment, increased potassium supply depressed N A R, on the average of all treatments, but showed even more complex interactions with the other nutrients than in the early periods. This variability in the effect of potassium is puzzling, and no satisfactory explanation of it can be offered. If data on the composition of the leaves at successive stages of growth were available, N A R might be found to be related more consistently to the potash content of the leaves than to the external supply of potassium. A possible explanation of the interactions between nutrients in the later stages of the barley experiment is that deterioration of the photosynthetic mechanism of the leaves during the phase of ripening of the plant began earlier or proceeded more rapidly in plants with an unbalanced nutrient supply, but such an hypothesis has little value unless the nature of the changes involved in this deterioration can be defined and their progress measured.

It is generally supposed that potassium is more directly concerned in the process of photosynthesis than the other nutrients. The only evidence of this in the present experiments was that the percentage increases of N A R and of leaf area calculated from the mean responses to potassium in the mangold

experiment (p. 397) were almost equal, whereas nitrogen and phosphorus had much greater effects on leaf area than on N A R. The mixture of sodium and magnesium salts behaved very similarly to potassium in its effects on N A R and leaf area in the mangold experiment, and this suggests that if potassium has special functions in relation to photosynthesis, these can, in the mangold, be fulfilled by sodium, for it is known (Watson, 1946) that application of sodium salts increases the sodium content of the leaves and reduces the potassium content. In the wheat experiment the sodium and magnesium salts apparently increased N A R, while potassium caused a small depression (Table VII). This might be taken to indicate that the magnesium increased N A R, if it is assumed that sodium, like potassium, was ineffective, but it would be unwise to accept this conclusion without supporting evidence, as the reliability of the estimates of the responses is doubtful, because they were derived from differences between single plots.

It has previously been supposed that the increase of yield produced by potassic fertilizer in the mangold experiment was mainly due to an increased assimilation rate of the leaves. Thus Russell (1937, p. 88) states that 'In series A, where ample but not excessive nitrogen is given, the weight of leaves is the same whether potassic fertilizer be given or not. But in the absence of potassium the leaves are only about half as effective, making only about half as much root as when it is given.' It has been shown (p. 397) that, in fact, the increased dry-matter production caused by potassic fertilizer in these conditions was almost wholly accounted for by increased leaf area; on the average of the whole period of observation, N A R was unaffected by the potassic fertilizer. The reason for the apparent discrepancy is that though potassic fertilizer increased leaf area at earlier stages of growth its effect decreased later and was negligibly small at the end of the growth period. Although potassium supply had no effect on the weight of leaves at harvest, it does not follow that the weight of leaves was unaffected throughout the whole period of growth.

Gregory (1937) gives tables which show that nutrient deficiencies reduced both maximum leaf area and maximum tiller number per plant in barley, the effects being in the order $N > P > K$. Potassium deficiency had little effect on meristematic activity as measured by tiller number, except when the level of supply was much reduced. The interactions between N, P, and K were positive; each of them had greater effects at high levels of supply of the others than at low levels. Similar results were found in the wheat and barley experiments (Figs. 1 and 2), where both nitrogen and phosphorus increased maximum shoot number per metre, but potassium and sodium had little effect. A point of difference is that in the barley experiment the P.KS interaction was negative both for leaf area and shoot number per metre (Table 5). This difference between the nutrients in their effects on meristematic activity was also apparent in the mangold experiment, where nitrogen and phosphate caused increases in the number of leaves per plant, but potassium and sodium had no effect.

SUMMARY

Growth analysis was used to investigate the physiological causes of variation in dry-matter yield produced by varied nutrient supply to crops of wheat, barley, and mangolds grown in 1939 on three of the classical field experiments at Rothamsted. In these experiments the same crop has been grown continuously on plots receiving varied annual fertilizer treatments, which have continued unchanged over a long period of years. Severe nutrient deficiency has developed on the plots where nutrients have been withheld, and the range of nutrient supply is consequently very wide. The experiments provide information on the effects of nitrogen in the form of sulphate of ammonia, phosphate, potash, a mixture of sodium and magnesium salts, farm-yard manure, and of fallowing during the previous year.

Nitrogenous fertilizer consistently increased net assimilation rate (N A R). This effect was not restricted to the later stages of growth and consequently cannot be attributed to delayed senescence of the leaves. In the early stages of growth nitrogen usually produced a greater increase of N A R in the presence of other nutrients than in their absence, but in the mangold experiment the addition of farm-yard manure or potash caused a reduction in the response to nitrogen.

Fallowing causes an accumulation of nitrate in the soil and, like nitrogen supplied in the form of fertilizer, it increased N A R.

The effects of the other nutrients on N A R were smaller and more variable. Phosphate and potash reduced the N A R of wheat, while sodium and magnesium salts apparently increased it. Up to the time of maximum leaf area, all treatments increased the N A R of barley, and the effect of each was increased by the presence of the others. In the subsequent period the average effects of nitrogen, phosphate, and a combined dressing of potassium, sodium, and magnesium salts were all negative, but the interactions between the nutrients were large, possibly because deterioration of the photosynthetic mechanism was more rapid in the leaves of plants with unbalanced nutrient supply in the senescent phase preceding death of the whole plant. In the mangold experiment, phosphate, potash, and the mixture of sodium and magnesium salts all increased N A R in the period before maximum leaf area. Subsequently, the interactions were large compared with the main effects, as in the barley experiment, but it is doubtful whether this has any connexion with senescence or whether it was merely the result of increased sampling errors. In this period phosphate markedly reduced N A R, while potassium or sodium and magnesium salts increased it, when applied separately, but reduced it when applied together.

All treatments increased leaf area per metre or per plant, but the time at which the effects occurred, and the manner in which they were produced, differed with the different nutrients. In the cereal experiments, nitrogen increased both shoot number per metre and leaf area per shoot, so that its effect on leaf area per metre was apparent throughout the whole growth period.

Phosphate increased shoot number, but had little effect on leaf area per shoot, tending to depress it in the later stages, presumably by hastening the death of the leaves. Potassium and sodium, on the other hand, had little effect on shoot number, but increased leaf area per shoot, possibly by prolonging the life of the leaves. Consequently, phosphate produced its greatest effect on leaf area per plant at an earlier stage of growth than potassium or sodium. In the mangold experiment nitrogen increased both leaf number and leaf size, and its effects continued to increase throughout the whole period of growth. The increases of leaf area produced by phosphate, potassium, and sodium were maximal in August and September, later falling to zero in October. Phosphate increased both leaf number and leaf size, but potassium and sodium had no effect on leaf number.

The effects of farm-yard manure were similar to those of a fertilizer mixture supplying nitrogen, phosphate, and potash.

In general, the effects of varied nutrient supply were relatively greater on leaf area than on N A R; variation in dry-matter production caused by varying nutrient supply was mainly the result of changes in leaf area, and variation of N A R was of secondary importance. In some conditions, however, potassium and sodium produced a greater percentage increase in N A R than in leaf area. This may imply that potassium has special functions in relation to photosynthesis; if so, it appears that in mangolds these functions can also be fulfilled by sodium.

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Studies in the Development of the Inflorescence

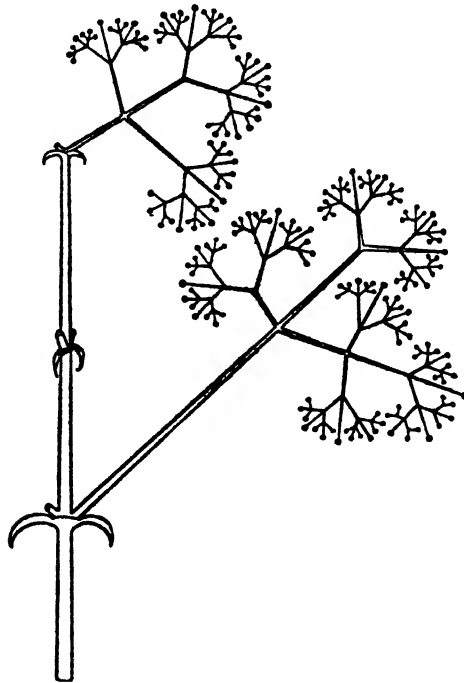
III. The Thyrses of *Valeriana officinalis* L.¹

BY

W. R. PHILIPSON

With Plate III and four Figures in the Text

THE habit of *Valeriana officinalis* L. has been described fully by Sprague (1944). The leaves are frequently arranged distichously on young shoots, but on stronger stems they are decussate, and this is always so in the region of flowering. The flowers are arranged in dichasial clusters in the axils of the



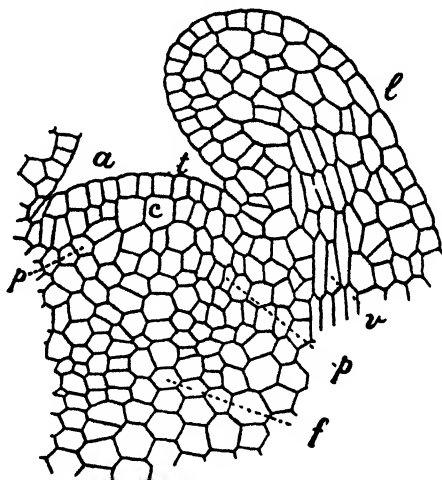
TEXT-FIG. 1. Diagram of the upper flowering branches of *Valeriana*. All the branchlets are represented as lying in one plane, but in nature are decussate. Note the frequent occurrence of dichotomy in both the major and minor branches.

upper leaves or bracts (Text-fig. 1). The lengths of the peduncles of the upper clusters are commonly adjusted so as to bring them into one terminal group, but the lower pairs of flower-clusters, especially in strong plants, remain distinct.

The structure of the apical meristem at a time when it is giving rise to

¹ Part of a Thesis approved for the degree of Ph.D. in the University of London, 1947. [Annals of Botany, N.S. Vol. XI, No. 44, October, 1947.]

opposite leaves is shown in Plate III, Fig. 1, and the disposition of the cells in this apex is indicated in Text-fig. 2. The cells of the youngest pair of leaf primordia (Text-fig. 2, *l*) have begun to mature, especially on the abaxial side, leaving meristematic tissue at the tip and at the surface towards the stem apex. The right-hand primordium is cut medianly, and in it a provascular meristem (*v*) is visible. Between these two primordia is the very small apex

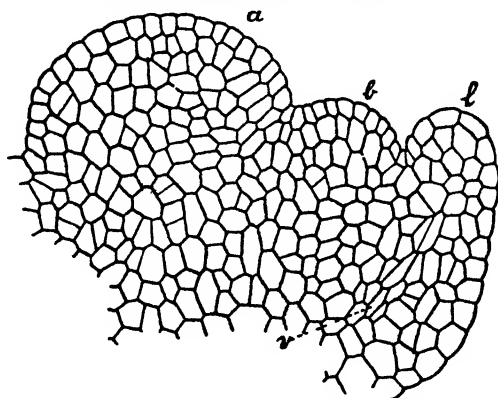


TEXT-FIG. 2. Outline drawing of the cells of the apex of the leafy shoot represented in Pl. III, Fig. 1. *a*, apex; *c*, central zone of corpus; *f*, file meristem; *l*, primordium of leaf; *p*, peripheral zone of corpus; *t*, tunica; *v*, provascular meristem of leaf.

(*a*), which is covered by a single tunica layer (*t*). Within the tunica the corpus displays the following zones: (i) a central initiation zone of large comparatively lightly staining cells (*c*), and (ii) a peripheral zone (*p*) of smaller more deeply staining cells which surround the initiation zone. Below the apical meristem a file meristem (*f*) develops. As is usual in vegetative apices, the formation of axillary-bud primordia lags behind that of the subtending leaves. No distinct bud can be distinguished in the general mass of meristematic cells in the axils of the youngest leaf-pair. Pl. III, Fig. 2, represents a median section through the base of one leaf of the third youngest leaf-pair (which lies in the same plane as the two youngest primordia). The mid-rib of this leaf is cut at (*m*), and the sheath connecting the primordia of the second pair of leaves at (*s*). No bud primordium has formed in the axil of the third leaf, but a small number of cells (*b*) have retained their meristematic nature. This delayed development of axillary buds is in contrast with the condition to be described in the inflorescence.

A general view of an apex of a shoot of *Valeriana* which is developing into an inflorescence is represented in Pl. III, Fig. 3, as seen under the low power of the microscope. The greater development of the internodes as compared with the vegetative apex gives the stem apex a more steeply conical form and

the axillary buds are noticeably developed. A section through the same apex passing medianly through one of the most recently formed bract primordia is seen under the high power in Pl. III, Fig. 4, and the disposition of the cells is represented in Text-fig. 3. The primordium (Text-fig. 3, *l*) is at an earlier stage of development than that in Text-fig. 2, being smaller and showing only the slightest indication of a central provascular meristem (*v*). The position of the primordium relative to the apex is very different and this manifestly is due to the development in its axil of the bulky primordium of an axillary bud (*b*). The structure of the apex itself also differs from that of the vegetative apex. It is enveloped by a single tunica layer, but no differentiation



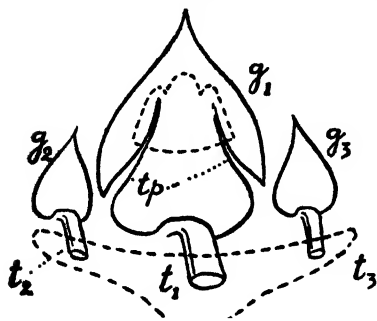
TEXT-FIG. 3. Outline drawing of the inflorescence apex represented in Pl. III, Fig. 4. *a*, apex; *b*, bud of flowering branch; *l*, primordium of bract; *v*, provascular meristem of bract.

of its cells into zones can be discerned; the apex is crowned by a uniform meristem which resembles the peripheral meristem of the vegetative apex in the size and staining properties of its cells, rather than the central zone.

The initiation and early development of the bract primordia and of the buds in their axils closely resembles that described for *Dipsacus* (Philipson, 1947). That is to say, the primordium of a bract develops by divisions in the sub-epidermal cells, and its provascular meristem forms acropetally by the progressive maturation of the surrounding cells of the primordium. The bud primordium develops from cells in the axil of the bract primordium; these cells retain their meristematic character and as soon as the bract primordium is visible they divide rapidly to form the projecting bud. The bud primordia form isolated pockets of meristematic tissue completely surrounded, except to the outside, by vacuolated tissue. Each develops into one of the cymose clusters of the mature inflorescence; the development of the inflorescence, therefore, consists of the summation of the development of these buds, and it will suffice if the development of one is described, together with any modifications which may be found at the extreme apex of the inflorescence.

The axillary meristems which develop into the lateral branches of the inflorescence originate, as has been stated, from cells of the apical meristem which retain their meristematic properties. The cells of these axillary meristems

show the same disposition into a single layered tunica enclosing a less regularly arranged corpus as is found in the apex of the inflorescence rudiment. By the active division of all its cells the axillary meristem comes to protrude as a dome-shaped primordium (Pl. III, Fig. 3, *b* in median longitudinal section (low power); Pl. III, Fig. 5, in tangential longitudinal section (high power)).



TEXT-FIG. 4. Diagram of the vascular system in the axil of a bract. The bract and the primordium of the flowering branch are indicated by broken lines. g_1 , g_2 , g_3 , leaf-gaps corresponding to t_1 , t_2 , t_3 , the three traces of the bract. tp , the traces of the flowering branch.

At this stage the primordium consists entirely of meristematic tissue, with no sign of lateral appendages or provascular meristems; it is not, however, totally devoid of differentiation, for it is possible to discriminate an apical meristem (*a*) from a file meristem (*f*), and at the base of the bud there is an intercalary meristem (*i*) in which repeated transverse divisions evidently occur by which the height of the bud is increased.

When the bud primordia are about $120\ \mu$ high, periclinal divisions in the layer below the tunica lead to the projection of the primordia of the prophylls. Simultaneously with the appearance of these primordia, provascular meristematic strands

which will develop into their mid-traces differentiate from the general meristem of the bud and become connected with the provascular meristem of the main axis of the inflorescence by the appearance of meristematic strands in the intervening vacuolated cells (cf. floral traces in *Dipsacus*, Philipson, 1947). Pl. III, Fig. 6 represents the bud of a lateral branch of the inflorescence of *Valeriana* cut in tangential longitudinal section, that is, across the axil of the subtending bract and through the two prophylls (*p*). The provascular strands of the mid-ribs can be seen for some distance below both primordia, but they leave the plane of the section before they make contact with the stele of the main axis. A reconstruction of the vascular system of a bud at this stage of development is represented diagrammatically in Text-fig. 4. The three traces (t_1 , t_2 , t_3) of the bract each leave the stele of the main axis by a separate gap (g_1 , g_2 , g_3). The traces of the prophylls of the axillary bud (tp) are inserted directly on the strands which border the gap corresponding to the mid-rib of the bract. At a later stage in its development the lateral branch will possess a cylindrical stele, but this will nevertheless resolve itself into two transverse strands at its insertion on the main axis, and these strands correspond with the mid-ribs of the prophylls. Pl. III, Fig. 7 represents a transverse section through a lateral branch in which a cylindrical stele (*a*) has been developed; a section taken lower on the same inflorescence (Pl. III, Fig. 8) shows the stele of the lateral branch divided into two strands (tp) which are about to unite with the stele of the main axis (*A*). The three strands of the subtending bract are lettered t_1 , t_2 , t_3 . The strands visible in the first photo-

graph on either side of the mid-rib are not inserted independently on the stele.

Once the primordia of the prophylls have been formed the further development of the bud of a lateral branch closely resembles that of the apex of the main axis of the inflorescence. That is to say, buds of a second order immediately appear in the axils of the prophylls, and the prophylls are followed by further pairs of bracts which are arranged decussately along the lateral branches. Pl. III, Fig. 9 represents a median longitudinal section of an inflorescence rudiment the lateral buds of which are about to produce buds of a second order. In Pl. III, Fig. 10 one of the lateral buds is shown cut in tangential section so that the prophylls and secondary buds are cut medianly. The resemblances between the apex of this lateral branch and that of the main axis (see Pl. III, Fig. 4) are very close. In the lateral bud shown to the left of Pl. III, Fig. 11, the second pair of bracts subtend well-developed buds. The further development of the inflorescence consists of a repetition of the processes already described, with the modification that the later-formed lateral branches of the main axis are progressively smaller, as are those produced successively upon each lateral branch, and also that a similar gradation in the size of the subtending bracts occurs. Indeed, the buds come more and more to dominate their bracts, but no bud has been seen to precede the primordium of its bract, as described by Warming (1872). The result of this continued distichous branching is shown in Text-fig. 1, which represents diagrammatically a typical inflorescence. It will be seen from this diagram that the typical branching previously described frequently becomes modified by the occurrence of dichotomy in both the main and the lateral axes. The early appearance of the lateral buds in the inflorescence has already been described; eventually they arise so early and involve so much of the apical meristem that no residuum of the apex remains between them to continue the main axis (see Pl. III, Fig. 12).

Finally a stage is reached when the buds, instead of dividing further, become floral primordia. This process has not been traced in detail, but its inception may be recognized by the flattening of the apex of the buds and the elevation of the periphery to form a shallow cup.

DISCUSSION

The inflorescence of *Valeriana* differs from those treated in previous papers of this series (Philipson, 1946; 1947) in the absence of an abrupt break between the vegetative and reproductive regions of the axis. A gradation can be recognized between these regions, for the leaves became progressively reduced and the lateral buds successively more copiously branched. In strong plants the gradation is particularly noticeable, for in them buds of some upper foliage leaves may develop into weak flowering branches. These two opposing gradients—a diminution of foliage and an accentuation of flower production—have been described in the young inflorescences of grasses (Evans and Groves, 1940) and are discussed by Prat (1945). A 'dilution of the tendency to leafiness'

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in successive sepals of the rose is described by Bond (1945) and was considered to be controlled, in all probability, by hormones. Morphological gradients of this nature have long been recognized, and have often been regarded as reflecting physiological changes within the plant; this view-point was clearly stated so long ago as 1790, when Goethe published his essay on the metamorphosis of plants, for he regarded the formation of floral structures to be due to the progressive 'purification' of the sap, a process that could be retarded or reversed by cultural treatment. A restatement in modern terms of the view that the production of floral organs is governed by internal gradients is given by Thompson (1944).

A purely morphological investigation cannot disclose the internal changes which underlie these two external gradients, but it was thought that it might be possible to relate them to some previous morphological change at the stem apex. For example, the replacement of the central zone in the meristem of vegetative apices by an extension of the peripheral zone to form the 'manchon' of the reproductive apex precedes the formation of flower primordia in *Bellis*, *Succisa*, and *Dipsacus* and the formation of the buds of flowering branches in *Valeriana*. This transformation from the vegetative to the reproductive type of apical meristem may, therefore, be related to a physiological change equivalent to the suspension of the supply of auxin by the apical meristem, with a consequent release of the dormant lateral meristems. It may be that the reproductive apex does not produce auxin, its place being taken by a flower-producing hormone (Chailakhaian, 1937).

The inflorescence of *Valeriana* is interesting, for its system of branching is midway between that of a raceme and a cyme. The distinction between these two classes of inflorescence, depending on the number of lateral branches developed and the extent of their further branching, is inevitably one of degree, allowing of a gradual transition from one class to the other (see Parkin, 1914, and Goebel, 1931, for diagrams illustrating this transition in *Campanula*). Speaking broadly, it may be said that in a cyme the lateral buds are inhibited until the apex of the main axis is 'determined', whereas in a raceme they are released while elongation of the main axis is still in progress. This growth may even be mainly vegetative, resulting in a leafy shoot with axillary flowers or inflorescences, as in the Soy Bean (Murneek and Gomez, 1936). In the cyme there appears to be a gradient from greater to lesser flowering down the axis, while in the raceme the gradient is reversed. These distinctions, depending on the degree of development of the lateral buds of the first, second, and higher orders, also appear to be of the kind governed by plant hormones, so that the study of the morphological classification of inflorescences approaches physiology.

The present investigation does not confirm the description and figures published by Warming (1872) of the formation in *Valeriana* of the floral primordia before those of the corresponding bracts. Nor do the bracts and buds arise from a common primordium, as Grégoire (1938) considers to be the case in *Delphinium*, but from distinct proliferations of the layer below

the tunica, as in *Dipsacus* (Philipson, 1947). Grégoire considered the close union of bract and flower to be a feature distinguishing them from the vegetative leaf and bud, and cited Warming in support of this view, though Warming had in fact emphasized the close union of the bud with both leaf and bract.

The division of the axis above the highest bract primordia into two equal branches is an example of true dichotomy, the occurrence of which in inflorescences where buds develop simultaneously with their bracts was noted by Warming. This type of dichotomy is not infrequent in the inflorescences of plants with opposite bracts and is due to the primordia of the flowering branches developing closer and closer to the apex, until the two buds take up all the meristematic tissue at the apex. It bears no relation to the dichotomy described by Stout (1941) in the inflorescence of *Hemerocallis*, which is due to the coalescence of lateral branches with the main axis. In plants with spiral bracts this tendency may lead to the formation of terminal buds (Aber, 1931).

SUMMARY

The features of the stem apex of *Valeriana officinalis* L. during the production of foliage leaves are described. The apical meristem comprises a single tunica layer enclosing a corpus in which three zones are recognized, namely, (i) a central initiation zone; (ii) a peripheral zone; and (iii) a rib meristem. The shoot apex during the production of bracts shows a similar single tunica layer, but no indication of a central zone is present in the corpus. The development of the bracts and their buds is essentially the same as that of foliage leaves and buds, but in contrast to the buds in the axils of foliage leaves those in the axils of bracts develop very early. They originate on the apex above the highest pair of bracts and quickly form prophylls and buds of the second order. Ultimately the two buds comprise the whole of the apex, leaving no residuum to continue the growth of the axis. The development of the lateral branches closely resembles that of the main axis. Morphological gradients between leaf and flower production are recognized and their possible dependence on hormones discussed. It is suggested that the distinction between the cyme and the raceme may depend on growth patterns controlled by hormones.

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DESCRIPTION OF PLATE

Illustrating Dr. W. R. Philipson's article 'Studies in the Development of the Inflorescence. III. The Thyrses of *Valeriana officinalis* L.'

Fig. 1. Longitudinal section through stem apex. For explanation see Text-fig. 2. ($\times 165$.)

Fig. 2. Longitudinal section through the axil of a leaf at the third node below the apex. *b*, residual meristematic cells in position of bud primordium; *m*, provascular meristem of mid-ribs; *s*, sheath joining the two leaves of the second node. ($\times 165$.)

Fig. 3. Longitudinal section through a young inflorescence. *b*, primordium of a lateral flowering branch. ($\times 60$.)

Fig. 4. Longitudinal section through the apex of an inflorescence at the stage shown in Fig. 3. For explanation see Text-fig. 3. ($\times 165$.)

Fig. 5. Tangential longitudinal section through a flowering-branch primordium at the stage seen at (*b*) in Fig. 3. *a*, apical mass of meristematic cells; *f*, vertical rows of meristematic cells; *i*, region of intercalary meristem. ($\times 165$.)

Fig. 6. Tangential longitudinal section through a flowering-branch primordium at a more advanced stage than that shown in Fig. 5. The paired primordia of the prophylls (*p*) have been formed. ($\times 165$.)

Fig. 7. Transverse section through the main axis of an inflorescence, a bract, and the base of the flowering branch in its axil. *s*, the circular stele of the flowering branch; *t*₁, *t*₂, *t*₃, the traces of the bract. ($\times 45$.)

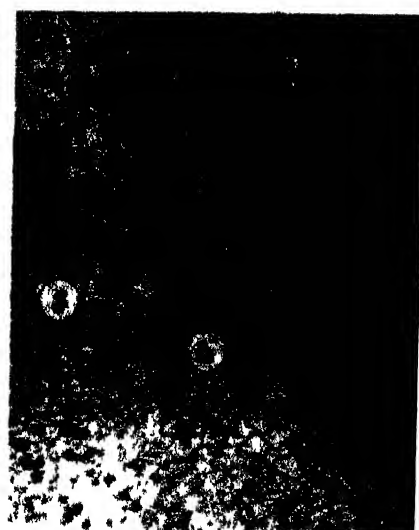
Fig. 8. Transverse section through the same inflorescence as that in Fig. 7, at a lower level. *A*, the stele of the main axis; *t*₁, *t*₂, *t*₃, the traces of the bract; *tp*, the two traces from the flowering branch which are about to unite with the strands bordering the leaf-gap corresponding to the mid-rib of the bract. ($\times 45$.)

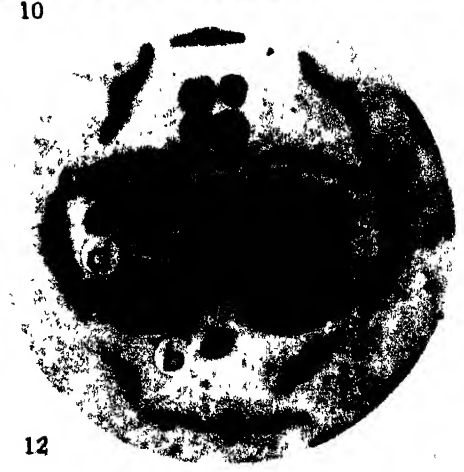
Fig. 9. Longitudinal section through an inflorescence the lateral branches of which are branching again—see Fig. 10. ($\times 60$.)

Fig. 10. Tangential longitudinal section through a flowering-branch primordium taken from the same inflorescence as that shown in Fig. 9. *b*, bud in axil of prophyll. ($\times 165$.)

Fig. 11. Longitudinal section through an inflorescence in which buds have formed in the axils of the first pair of bracts above the prophylls of a lateral flowering branch. ($\times 68$.)

Fig. 12. Transverse section through the ultimate branches of an inflorescence showing the formation of a pair of axillary buds without any residue of the main axis remaining. *b*, one of the highest pair of bracts; *d*, the two terminal buds forming a dichotomy of the main axis. ($\times 45$.)





The Gaseous Exchange between the Root and the Shoot of the Seedling of *Cucurbita pepo*

BY

R. BROWN

(Botany Department, The University, Leeds)

With three Figures in the Text

INTRODUCTION

THE object of this work has been the elucidation of a chance observation of an unexpected kind, namely, that in a normal intact seedling of *Cucurbita* in which both the root and the shoot are exposed to air, nitrogen is continuously lost from the root in considerable quantities, but the outflow ceases abruptly on decapitation of the seedling. In the course of the inquiry it was also found that with suitable experimental provision an outflow of hydrogen and oxygen from the root occurred when these gases were supplied to the shoot but not to the root; and again the outflow ceased when the seedling was decapitated. The effect of decapitation was undoubtedly that of interrupting a gaseous stream that flowed to the root, and it is significant that it was found that the effect of decapitation by severing the hypocotyl was replaceable by the removal of the cotyledons only. Moreover, the flow of gases downward was also stopped by severing the root. Simultaneously, with the downward movement of gases from cotyledon to root a movement of a different kind in the reverse direction was also detected. Explanation of all these facts in terms of current concepts of simple diffusion of gases through the intercellular space system of the plant proved to be completely inadequate, and it seems necessary to postulate the existence of some mechanism for the active translocation of the gases in solution. The exact path of flow has not yet been determined but the experimental evidence, as far as it goes, points to either the parenchyma or the vascular tissue.

Since the experimental method for accurate assessment of gaseous exchange by the root of a growing plant is an essential part of the inquiry, the design and use of the apparatus will be described in some detail. An assessment of sources of error will follow and the results obtained can then be most conveniently expressed in tabular form and only the interpretation discussed in detail.

METHODS AND MATERIALS

If gaseous exchange occurs between the root and the shoot, then any treatment applied to the shoot which affects the general relation between it and the root should, of course, influence the exchange between the root and the

local environment. This consideration forms the basis of the general technique that has been used in this investigation, and it involves an estimation of the gaseous exchange of the root in terms of absolute quantities of oxygen, carbon dioxide, and nitrogen. The apparatus designed for the purpose is shown diagrammatically in Fig. 1, and is described below in relation to the root chambers A_1 and A_2 , the pump C , and the general design of the apparatus.

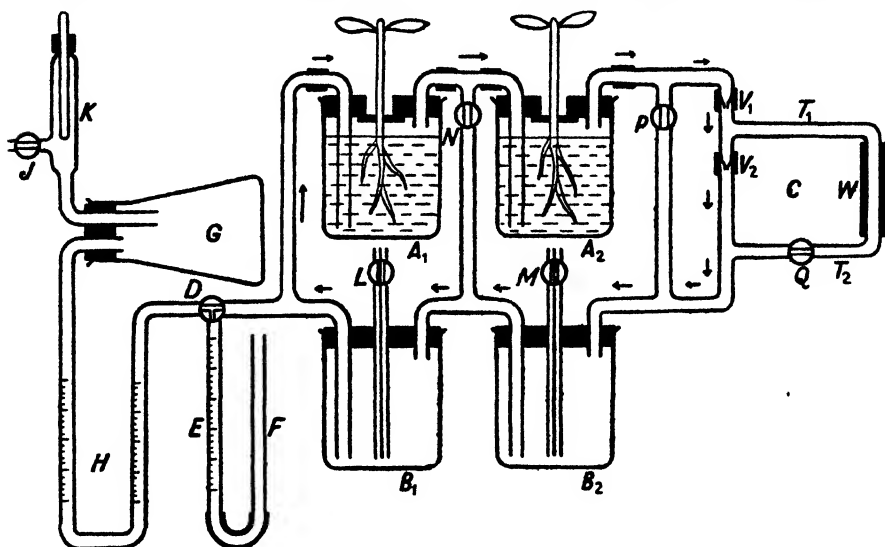
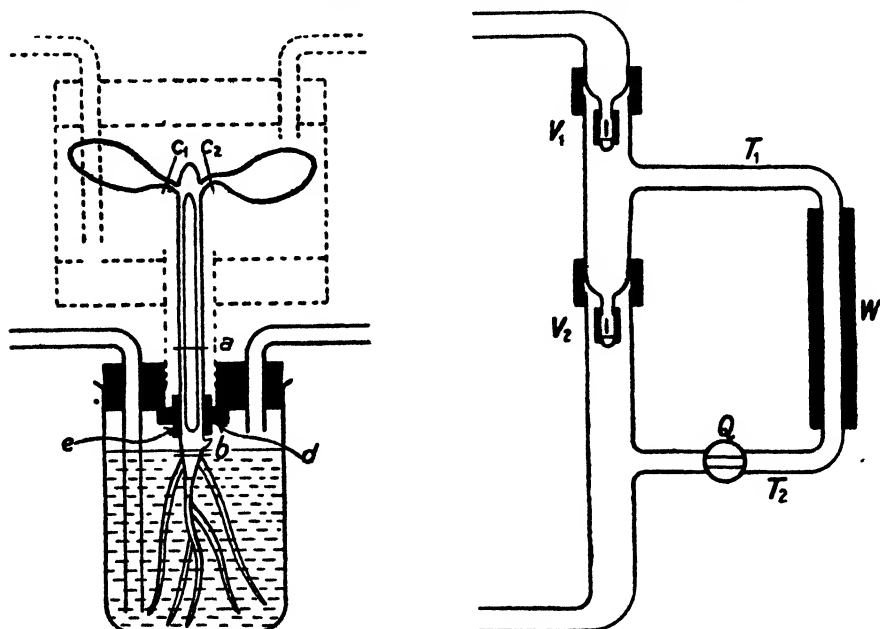


FIG. 1. Diagram of apparatus for determining gaseous exchange of root.
For explanation see text.

The root chamber is shown in greater detail in Fig. 2. In the present connexion the component shown in broken outline may be disregarded. The vessel that holds the seedling consists of a short specimen-tube closed by a rubber stopper provided with inlet and outlet tubes for the gas stream, and with a special device in the centre for holding the hypocotyl in a gas-tight seal. The rubber stopper, which is 2.5 cm. in diameter, is traversed by a central channel about 1 cm. in diameter. A rubber disc, d , which is 2 mm. thick and perforated by a circular hole 3 mm. in diameter, is attached with rubber solution to the lower surface of the stopper. The hypocotyl which is provided over a length of about 1 cm. from the upper extremity of the root with a thin rubber sleeve, e , is gripped in the central hole of the disc. The sleeve is fitted on to the hypocotyl by stretching over a length of 9-mm. glass tubing from which it is released on to the hypocotyl held in the centre of the tube. A similar tube is made to stretch the central hole of the disc, d , and then withdrawn with the hypocotyl again held in the centre of the tube and with the sleeve opposite the disc. By this device the necessary contact with rubber over a large surface is secured with little pressure, and the dangers of crushing involved in the use of a split stopper and of infiltration of the tissues with luting greases are avoided.

Two root chambers are incorporated in the circuit. They each contain about 40 ml. of water in which the roots are immersed, and through which the gas stream is passed.

The pump at c of Fig. 1, part of which is shown in more detail in Fig. 3, is based on a design developed by Fernandes (1923). The two tubes (T_1 and T_2) are attached to a length of thick-walled pressure tubing, w, which is



FIGS. 2 and 3. Fig. 2. Diagram of root chamber with plant in position.
Fig. 3. Diagram of part of the pump shown in Fig. 1. For explanation see text.

alternately compressed and relaxed in a device not shown in Fig. 3, but consisting of a base plate and an upper plate, the latter of which is secured to two metal rods, which are pulled upwards by springs attached to lateral supports and thrust downwards by rotating cams. Each of the valves V_1 and V_2 consists of a short length of narrow rubber tubing, slit longitudinally along one side in a central zone, with one end closed by a piece of glass rod and the other stretched over the tapered end of a tube in the gas circuit. The valve fits loosely in a second tube which continues the circuit, the two tubes being held together by a short length of pressure tubing which is stretched over both.

The gas circuit follows the course of the arrows in Fig. 1; it traverses the two root chambers A_1 and A_2 and the two vessels B_1 and B_2 , which are inserted to accommodate the relatively large volume of gas which must be provided to compensate for the large volume of water present in A_1 and A_2 . Both B_1 and B_2 are provided with rubber stoppers traversed by inlet and outlet tubes and by a third capillary tube carrying a tap through which gas may be withdrawn for analysis. Through the tubes carrying the taps, N, P, and Q, local pressure

differences due to the action of the pump are dissipated and the whole system brought to uniform pressure. The gas circuit is connected through the 3-way tap D with a manometer H, containing paraffin, and with a tube E, graduated in units of 0.01 ml., which is connected by a short length of rubber tubing with a mercury reservoir F. The several vessels of the gas circuit are immersed in a water-bath maintained at 25° C.; B₁ and B₂ wholly so, and A₁ and A₂ only up to the rims, with the tubing on the side of B₁ and B₂ immersed, and that on A₁ and A₂ exposed to the fluctuating temperature conditions of the atmosphere above the bath. The consequent fluctuation in pressure on the right-hand side of the manometer H is compensated by the device attached to the left-hand limb, consisting of a conical flask G of about the same volume as the immersed components on the right-hand side of H and which is itself immersed, and a tube connected to G of about the same volume as the exposed components of the system on the right-hand side of H, and which is itself exposed. The free end of K tapers to a sleeve traversed by a glass rod to which it is secured by a short length of pressure tubing stretched over both. By this arrangement the volume of K may be varied by adjusting the length of the glass rod in K. K is also provided with a side arm carrying the tap J through which the left-hand limb of H may be connected to the atmosphere. K is not operated in the position shown in Fig. 1, but is placed between A₁ and A₂ and B₁ and B₂ at about the same level above the bath as the exposed portions on the side of A₁ and A₂.

The gaseous exchange between the root and the atmosphere to which it is exposed is determined by measuring the change in the volume of each of the gases in the mixture circulated through the water in which the roots are suspended. This involves measuring (a) the volume of the gas system at the beginning of the experiment, (b) the volume of the same system at the end of the experiment, and (c) the change in the composition of the gaseous mixture in the course of the experiment. The procedure for each of these experimental phases is described separately below.

For the measurement of the initial volume of the gas system the right-hand limb of H is connected to E and with the system of the gas circuit, the taps J, N, P, Q, L, and M are opened, and mercury is brought to the highest mark in F. L and M are closed, F is lowered, and the resulting decrease in pressure in the gas circuit is measured from H. In order to calculate the volume in addition to the change in pressure the change in volume must also be known. This is given by the change in level in E less the volume occupied by the rise of fluid in the right-hand limb of H. The internal volume of the gas circuit is about 200 ml.

When the initial volume has been determined, all parts of the system are brought to atmospheric pressure by opening all taps on both sides of the manometer. At this stage the circulation of gas over the roots can begin. J, N, P, Q, L, and M are closed, the level of mercury in E is noted, and the pump started. At the end of the experimental period the volume of the gaseous system in the circuit is again determined, but on this occasion by determining

the change in volume and adding this to the initial volume. For this purpose, after the pump has been stopped with the pressure tube *w* in the relaxed position it was in at the beginning of the experiment, *N*, *P*, and *Q* are opened and *F* is either raised or lowered until the level of manometer fluid in the two limbs of *H* is the same. Since the plants transpire during the experiment and the volume of water is consequently reduced, it is evident that the change in pressure during the experiment is not due to change in volume in the gas circuit alone; and the change in level in *E* is therefore due to changes in the volumes of gases and of water. Thus to determine the volume decrease in the gaseous component it is necessary to determine separately the amount of water lost by transpiration, and adjust the volume indicated by the level change in *E* by this amount. The water transpired is determined by weighing the two root chambers before the circulation of the gas begins and again at the end of the experiment.

The change in volume of the gaseous phase added to the initial volume gives the total volume of the gaseous mixture at the end of the period of observation. This indirect method of estimating the final volume is necessitated by the high error involved in the direct method used in estimating the initial volume. When estimating this quantity from the change in pressure with increase in volume duplicate determinations may differ by as much as 5 ml., while the change in volume is rarely more than about 0.5 ml. The indirect method of estimating the final volume avoids obscuring the difference by the errors of two direct estimations.

A sample of gas is withdrawn through *L* and analysed in a Haldane gas-analysis apparatus before the root chambers are removed for the determination of the loss of water by transpiration.

The comparison of the results of this analysis with the known composition of the mixture when it is introduced into the apparatus gives, of course, the change in composition that results from the activity of the root.

From the volumes of the gaseous phase at the beginning and end of the experimental period and from the known composition of the mixtures at the two stages the change in the volume of each component is calculated. The gaseous mixture is in equilibrium with the water in the two root chambers through which it is circulated, and since the volume of water (about 80 ml.) is large and the percentage change in composition with respect to certain components of the mixture is also relatively large, the quantities of each component in solution at the beginning and end of the experiment are calculated and added to the corresponding values for the gaseous phase. In calculating the volume of each gas in solution the changes in pressure, whether due to changes in composition of the gaseous mixture or to the action of the pump, have been disregarded, since in the aggregate they do not exceed 4 mm. of mercury.

The experimental procedure outlined above is that involved in making a single set of observations in a series. In the experimental design adopted six consecutive observations are made with the same pair of plants. In most

experimental series the first three observations are made with intact plants and the last three with the same pair after experimental treatment. Gas is circulated over the root on each occasion for 1 hour and the interval between one period of circulation and the next is 30 minutes, during which time the operations for estimating the change in volume and composition of the gas are made. During the interval the gases left in the system at the end of the previous experimental period are expelled and replaced by the original mixture.

Errors. These are readily determined from certain of the data given in the next section. The last three results in each series given at the bottom of Table II are obtained in a system in which an isolated root is immersed in the water in the root chamber and in which the hole in the rubber disc of the stopper which is normally occupied by the hypocotyl in this instance is closed with a short length of glass rod. The data have been calculated by the method outlined above, and apparent variations in the nitrogen content of the system, therefore, represent the errors of the estimation. From the values given it is evident that the estimation of nitrogen is subject to an error of ± 0.05 ml. The error is large and is indeed sufficiently large to make the differences between successive nitrogen values for intact plants of doubtful significance. They are not sufficiently large, however, to obscure the dominant effects of shoot treatment. Moreover, since nitrogen is the only gas estimated entirely by difference, it is improbable that the values calculated for other gases are subject to a larger or even as large an error.

When the root is attached to the hypocotyl and this extends through the rubber stopper of the root chambers, two possible further sources of error may be considered: errors arising from the determination of the water withdrawn from the root chamber and those from a possible inadequate seal between the hypocotyl and the rubber components by which it is gripped. The transpiration loss may not represent all the water withdrawn from the root chamber, since some of this may be retained in the shoot as a result of growth. An estimate of the water retained by the whole seedling has been obtained by comparing the change in weight of the whole system with the change in weight of the water alone over a period of an hour, and the difference is never greater than 0.01 ml. Although the experimental gases are being continuously circulated through the water in the root chamber, and some loss through removal of water vapour in the gas stream may occur, this is avoided completely by ensuring that the gas stream is saturated with water vapour before the experiment begins.

Although it is difficult to detect leaks in a system which is exposed to a free water surface, the data, and particularly those of Table VIII, indicate that an effective seal between the hypocotyl and the rubber stopper of the root chamber is in fact established. In the experiments of Table VIII, after a preliminary period of observation with the intact plant, the seedling is decapitated by cutting across the hypocotyl at a point outside the root chamber, so that after the operation the root is attached to a short length of

hypocotyl which extends into the atmosphere external to the gas circuit. In these experiments cylinder nitrogen is provided for the root, with the shoot normally exposed to air. Nevertheless, after decapitation there is no leakage of oxygen inwards into the system.

The significance that may be attached to the nitrogen and to the carbon dioxide data given in the next section requires some comment. Since nitrogen is determined entirely by difference, there is no direct evidence that some of the gas so estimated is not some substance other than oxygen, carbon dioxide, and nitrogen. Such, however, is highly improbable since no gas is evolved from the root when air is supplied to the root and the shoot which reacts with oxygen when the gaseous mixture is drawn over a hot wire in the Haldane apparatus. Further, as shown below, hydrogen may flow to the root from the shoot, in which case a comparable flow of nitrogen might be expected.

The absolute values for carbon dioxide given in the next section are probably less reliable than the corresponding values for nitrogen and oxygen. The apparatus incorporates several rubber stoppers and several connexions made through rubber pressure tubing, and a loss of carbon dioxide through these by diffusion may occur. Since, however, the loss of carbon dioxide from this source would at all times be proportional to the concentration in the system, the error does not affect the relative significance of successive values.

Materials. For two reasons the experimental material used throughout has been seedlings of *Cucurbita pepo* at the stage just prior to the expansion of the first foliage leaf. The roots of these plants are pliable and are therefore easily manipulated without injury; secondly, the hypocotyl throughout its length is traversed by an air cavity which forms part of the intercellular space system of the plant, the significance of which for the internal movement of gases may be assessed by modifying experimentally the relation of the cavity with the external atmosphere.

RESULTS

With the apparatus designed for this investigation the atmospheres to which the root and the shoot are exposed are connected only through the tissues of the hypocotyl, and the effect of the separate atmospheres on the root exchange may therefore be determined. Although the largest number of experiments have been conducted with the same gaseous mixture, namely, air supplied to the two parts of the plant, some experiments have been conducted with some gases applied only to the shoot. Accordingly, two groups of results are described below, those obtained (A) with air applied to the root and the shoot, and (B) with different gaseous mixtures applied to the two parts of the plant.

The conditions that determine gaseous flow between the root and the shoot have been studied by comparing the root exchange of the intact plant with the exchange after various treatments have been applied to different organs. In each case three observations extending over the first $4\frac{1}{2}$ hours of the experiment are made with intact plants followed by three observations extending

over the final $4\frac{1}{2}$ hours after various excisions and mutilations have been applied. The various treatments whose effects have been studied are the following:

1. Isolating the root by cutting the connexion between it and the hypocotyl just below the rubber sleeve on the hypocotyl, which is subsequently withdrawn from the system and the hole in the disc of the stopper of the root chamber closed with a short length of glass rod. As a result of this adjustment all connexion between the root and the external atmosphere is severed.

2. The bulk of the root is withdrawn from the system by cutting across the root at *b* in Fig. 2. After this mutilation the change in gaseous compositions in the circuit is due only to the small root stump extending into the root chamber from the base of the hypocotyl.

3. The upper part of the shoot is removed by cutting across the hypocotyl at *a* in Fig. 2. The rest of the system is not disturbed, and consequently after decapitation a short length of hypocotyl extends through the stopper into the root chamber, carrying the root at its lower extremity. It is an important feature of the experimental situation that after decapitation the cavity of the hypocotyl is exposed to the atmosphere outside the root chamber, and since the cavity extends to the upper extremity of the root, this part of the root is brought into immediate contact with the atmosphere outside the root chamber.

4. A longitudinal slit is made in the hypocotyl at *a* in Fig. 2, the two segments being separated by a matchstick thrust between them. By this treatment the cavity of the hypocotyl is exposed to the atmosphere outside the root chamber, while tissue and vascular continuity is maintained between the shoot and the root.

5. The cotyledons are removed by cutting across the petioles at c_1 and c_2 in Fig. 2. After this treatment the hypocotyl is intact and remains exposed to the atmosphere outside the root chamber.

One phrase used in this and the next section requires some explanation. The ratio of carbon dioxide evolved to oxygen absorbed is of some significance for the interpretation of the data, but since the evidence suggests that some of the oxygen absorbed by the root of an intact plant may escape into the shoot, this ratio (CO_2/O_2) is designated the 'exchange quotient' instead of the respiratory quotient.

A. Experiments with air applied both to the root and the shoot

Table I gives the data for the basic demonstration for the evolution of nitrogen from the root when both root and shoot are in air. In these experiments the plants throughout the whole experimental period are not disturbed.

Table II provides a comparison of the root exchange with the intact plant and after isolation of the root in the circumstances detailed under (1) above. As a result of isolation the evolution of nitrogen ceases, there is an immediate increase in the value of the exchange quotient, an immediate decrease in oxygen uptake, and an immediate increase in carbon dioxide production.

After the immediate adjustment to isolation the rate of oxygen uptake and carbon dioxide production tend to decrease progressively with time, with a corresponding progressive increase in the value of the exchange quotient.

TABLE I

Results of two Experiments showing the Characteristics of the Gaseous Exchange of the Root when Root and Shoot are supplied with Air and the Plant is intact. (Values in columns 1, 2, and 3 represent gas (ml.) absorbed (—) or evolved (+) by two roots per hour; those in columns 4 the corresponding exchange quotients; and those in columns 5 water (ml.) transpired by the two seedlings per hour)

Hours.	Expt. 1.					Expt. 2.				
	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O
1.5	+0.17	-0.46	+0.33	0.72	0.12	+0.09	-0.59	+0.34	0.57	0.12
3.0	+0.19	-0.47	+0.36	0.77	0.12	+0.28	-0.71	+0.32	0.45	0.10
4.5	+0.19	-0.44	+0.36	0.82	0.12	+0.25	-0.53	+0.26	0.40	0.10
6.0	+0.19	-0.46	+0.36	0.78	0.12	+0.12	-0.36	+0.24	0.67	0.12
7.5	+0.17	-0.44	+0.36	0.82	0.12	+0.11	-0.45	+0.26	0.57	0.07
9.0	+0.10	-0.28	+0.36	1.28	0.14	+0.09	-0.19	+0.27	1.42	0.11

TABLE II

Results of two Experiments showing the Effect on the Gaseous Exchange of the Root of Separation from the Shoot when both Root and Shoot are exposed to Air, and when, after Separation, Connexion with the Atmosphere external to the Root Chamber is interrupted. (The first three values in each series relate to the intact plant, and the last three to the isolated root. Values in columns 1, 2, and 3 represent gas (ml.) absorbed (—) or evolved (+) by two roots per hour; those in columns 4 the corresponding exchange quotients; and those in columns 5 water (ml.) transpired by the two seedlings per hour)

Hours.	Expt. 1.					Expt. 2.				
	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O
1.5	+0.08	-0.62	+0.54	0.88	0.06	+0.13	-0.39	+0.16	0.41	0.09
3.0	+0.21	-0.65	+0.57	0.90	0.10	+0.08	-0.30	+0.24	0.80	0.18
4.5	+0.13	-0.65	+0.57	0.88	0.08	+0.11	-0.30	+0.21	0.70	0.18
	Roots isolated					Roots isolated				
6.0	-0.04	-0.59	+0.72	1.22	—	0.0	-0.27	+0.32	1.19	—
7.5	-0.01	-0.59	+0.75	1.27	—	-0.05	-0.15	+0.21	1.39	—
9.0	+0.04	-0.55	+0.54	0.99	—	-0.05	-0.15	+0.24	1.56	—

Table III shows the effect of decapitation by cutting across the hypocotyl at *a* in Fig. 2. The effect is similar to that of isolating the root. There is an abrupt cessation in the evolution of nitrogen, accompanied by an immediate decrease in the rate of oxygen uptake and probably an increase in the rate of carbon dioxide production; at the same time there is also an immediate increase in the value of the exchange quotient. With respect to the respiratory gases the immediate change is followed probably by progressive decreases in

both rates, and with a progressive increase in the value of the exchange quotient (this aspect of the position is shown more decisively by the data of Table X).

TABLE III

*Results of five Experiments showing the Effect on the Gaseous Exchange of the Root of decapitating the Seedling outside the Root Chamber (at *a* in Fig. 2) when Root and Shoot are exposed to Air. (The first three values in each series are obtained with the intact plant, the last three with the decapitated plant. Values in columns 1, 2, and 3 represent gas (ml.) absorbed (—) or evolved (+) by two roots per hour; those in columns 4 the corresponding exchange quotients; and those in columns 5 water (ml.) transpired by the two seedlings per hour)*

Hours.	Expt. 1.					Expt. 2.				
	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O
1.5	+0.19	-0.56	+0.37	0.66	0.16	+0.30	-0.50	+0.41	0.83	0.16
3.0	+0.21	-0.59	+0.39	0.66	0.16	+0.11	-0.42	+0.38	0.91	0.15
4.5	+0.21	-0.50	+0.39	0.78	0.14	+0.05	-0.38	+0.41	1.07	0.15
	Seedling decapitated					Seedling decapitated				
6.0	+0.01	-0.27	+0.24	0.89	0.04	-0.04	-0.26	+0.49	1.9	0.01
7.5	-0.03	-0.42	+0.42	0.99	0.06	-0.02	-0.49	+0.49	1.0	0.00
9.0	-0.04	-0.39	+0.39	0.98	0.06	-0.02	-0.37	+0.38	1.02	0.00

Hours.	Expt. 3.					Expt. 4.				
	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O
1.5	+0.14	-0.58	+0.50	0.87	0.39	+0.21	-0.63	+0.52	0.84	0.15
3.0	+0.19	-0.65	+0.66	1.01	0.36	+0.13	-0.58	+0.53	0.90	0.14
4.5	+0.44	-1.00	+0.66	0.66	0.36	+0.22	-0.58	+0.50	0.85	0.19
	Seedling decapitated					Seedling decapitated				
6.0	+0.02	-0.61	+0.74	1.19	0.01	+0.02	-0.48	-0.48	1.09	0.02
7.5	+0.01	-0.56	+0.66	1.19	0.03	-0.01	-0.39	-0.39	1.22	0.01
9.0	-0.05	-0.48	+0.64	1.32	0.02	+0.03	-0.39	-0.39	1.00	0.01

Hours.	Expt. 5.				
	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O
1.5	+0.19	-0.62	+0.57	0.93	0.11
3.0	+0.16	-0.58	+0.60	1.03	0.10
4.5	+0.10	-0.66	+0.57	0.86	0.09
	Seedling decapitated				
6.0	-0.02	-0.61	+0.83	1.35	0.01
7.5	+0.01	-0.61	+0.81	1.25	0.01
9.0	+0.01	-0.61	+0.59	0.97	0.02

Table IV shows the effect of removing the cotyledons at *c*₁ and *c*₂ in Fig. 2. The effect is indistinguishable from that of total decapitation.

Table V shows the effect of reducing the size of the root by cutting across it at *b* in Fig. 2. In spite of the persistence of an intact shoot the evolution of nitrogen ceases, with, of course, very considerable reductions in the rates of oxygen uptake and carbon dioxide production.

TABLE IV

Results of two Experiments showing the Effect on the Gaseous Exchange of the Root of removing the Cotyledons (at c_1 and c_2 in Fig. 2) when Air is supplied both to the Root and the Shoot. (The first three values in each series are obtained with the intact seedling, and the last three with the cotyledons removed. Values in columns 1, 2, and 3 represent gas (ml.) absorbed (—) or evolved (+) by two roots per hour; those in columns 4 the corresponding exchange quotients; and those in columns 5 water (ml.) transpired by the two seedlings per hour)

Hours.	Expt. 1.					Expt. 2.				
	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O
1.5	+0.17	-0.70	+0.54	0.77	0.12	+0.13	-0.73	+0.30	0.41	0.14
3.0	+0.22	-0.60	+0.48	0.80	0.12	+0.15	-0.61	+0.28	0.45	0.12
4.5	+0.28	-0.57	+0.41	0.73	0.12	+0.18	-0.58	+0.28	0.48	0.10
	Cotyledons removed					Cotyledons removed				
6.0	+0.05	-0.48	+0.61	1.24	0.04	+0.04	-0.49	+0.33	0.66	0.04
7.5	-0.05	-0.44	+0.37	0.85	0.06	+0.01	-0.46	+0.34	0.73	0.05
9.0	+0.03	-0.38	+0.42	1.10	0.02	+0.06	-0.46	+0.31	0.67	0.08

TABLE V

Results of two Experiments showing the Effect on the Gaseous Exchange of the Root of reducing to a Stump the Root within the Root Chamber when Air is supplied both to the Root and the Shoot. (The first three values in each series are obtained with the intact root, and the last three with the reduced root. Values in columns 1, 2, and 3 represent gas (ml.) absorbed (—) or evolved (+) by two roots per hour; and those in columns 4 water (ml.) transpired by the two seedlings per hour)

Hours.	Expt. 1.				Expt. 2.			
	1 N ₂	2 O ₂	3 CO ₂	4 H ₂ O	1 N ₂	2 O ₂	3 CO ₂	4 H ₂ O
1.5	+0.19	-0.62	+0.25	0.08	+0.08	-0.68	+0.66	0.08
3.0	+0.18	-0.44	+0.33	0.08	+0.09	-0.71	+0.66	0.06
4.5	+0.29	-0.47	+0.33	0.09	+0.16	-0.68	+0.64	0.07
	Root reduced				Root reduced			
6.0	+0.01	-0.01	+0.09	0.07	+0.01	-0.14	+0.17	0.08
7.5	+0.03	+0.01	0.04	0.07	+0.01	-0.17	+0.22	0.06
9.0	+0.07	+0.01	-0.08	0.10	+0.03	-0.15	+0.17	0.06

Table VI shows the effect of splitting the hypocotyl longitudinally so as to expose the central cavity and with it the air spaces of the plant to direct contact with the atmosphere around the shoot. As a result of this treatment the outflow of nitrogen from the root is unimpeded, but the exchange of the respiratory gases is at once altered. With respect to the respiratory gases the effect is similar to that of total decapitation: there is an immediate increase in the value of the exchange quotient accompanied probably by a decrease in oxygen uptake. The immediate effect is followed by a progressive decrease in the rates of oxygen uptake and carbon dioxide production, with progressive increase in the value of the exchange quotient.

TABLE VI

Results of two Experiments showing the Effect on the Gaseous Exchange of the Root of opening the central Cavity of the Hypocotyl to the Atmosphere when both Root and Shoot are in Air. (Values in columns 1, 2, and 3 represent gas (ml.) absorbed (—) or evolved (+) by two roots per hour; those in columns 4 the corresponding exchange quotients; and those in columns 5 water (ml.) transpired by the two seedlings per hour)

Hours.	Expt. 1.					Expt. 2.				
	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O
1.5	+0.07	-1.24	+0.84	0.68	0.07	+0.26	-0.68	+0.47	0.67	0.09
3.0	+0.25	-1.47	+1.02	0.69	0.08	+0.17	-0.66	+0.58	0.87	0.09
4.5	+0.28	-1.34	+0.96	0.72	0.08	+0.10	-0.72	+0.67	0.92	0.11
	Hypocotyl split					Hypocotyl split				
6.0	+0.16	-0.89	+0.79	0.88	0.08	+0.14	-0.73	+0.72	0.99	0.12
7.5	+0.10	-0.90	+0.75	0.84	0.09	+0.14	-0.64	+0.64	0.99	0.10
9.0	+0.22	-0.78	+0.75	0.97	0.10	+0.12	-0.56	+0.61	1.11	0.11

B. Experiments with different gaseous mixtures applied to the two parts of the plant

In the experiments of Table VII air is supplied to the roots and a mixture consisting of 20 per cent. hydrogen, 20 per cent. oxygen, and 60 per cent. nitrogen is supplied to the shoot, and drawn through the device shown by the broken outline in Fig. 2. The cotyledons are accommodated in a wide glass tube closed above by a rubber stopper traversed by inlet and outlet tubes for the gas stream and below by another rubber stopper traversed centrally by a tube which accommodates the hypocotyl and which is gripped in the central channel of the rubber stopper of the root chamber. In these experiments the observations are restricted to the detection and measurement of hydrogen in the atmosphere of the root, and the effect of decapitation by cutting across the hypocotyl at *a* in Fig. 2 is determined in relation to the evolution of hydrogen only from the root. The results of Table VII show that hydrogen is evolved from the root when the plant is intact, and that the outflow ceases immediately the seedling is decapitated.

TABLE VII

*Hydrogen (ml. per hour) released by two Roots of Plants, the Shoots of which are provided with an Atmosphere containing Hydrogen and the Roots with Air. (The first three values in each series relate to the intact plant, the last three to the plant with the upper part of the shoot removed at level *a* in Fig. 2)*

Hours.	Expt. 1.	Expt. 2.
1.5	0.13	0.07
3.0	0.18	0.07
4.5	0.11	0.09
	Shoot removed	Shoot removed
6.0	0.00	0.00
7.5	0.02	0.00
9.0	0.02	0.00

Table VIII shows the exchange before and after decapitation at *a* in Fig. 2 when the shoot is supplied with air and the root with cylinder nitrogen (containing 0.3 per cent. oxygen). While the seedling is intact oxygen flows out of the root and nitrogen is absorbed. After decapitation, however, the evolution of oxygen and the absorption of nitrogen cease abruptly.

TABLE VIII

Results of three Experiments showing the Effect on the Gaseous Exchange of the Root of removing the Shoot (at a in Fig. 2) when Cylinder Nitrogen is supplied to the Root and Air to the Shoot. (The first three values in each series relate to the intact seedling and the last three to plants with the shoot removed. Values in columns 1, 2, and 3 represent gas (ml.) absorbed (—) or evolved (+) by two roots per hour; those in columns 4 water (ml.) transpired by the two seedlings per hour)

Hours.	Expt. 1.				Expt. 2.				Expt. 3.			
	1 N ₂	2 O ₂	3 CO ₂	4 H ₂ O	1 N ₂	2 O ₂	3 CO ₂	4 H ₂ O	1 N ₂	2 O ₂	3 CO ₂	4 H ₂ O
1.5	—0.08	+0.06	+0.06	0.04	—0.56	+0.44	+0.25	0.16	—0.68	+0.50	+0.64	0.28
3.0	—0.08	+0.11	+0.06	0.06	—0.17	+0.14	+0.25	0.12	—0.24	+0.30	+0.16	0.28
4.5	—0.25	+0.30	+0.08	0.06	—0.15	+0.18	+0.17	0.14	—0.30	+0.24	+0.11	0.20
	Shoot removed				Shoot removed				Shoot removed			
6.0	—0.03	—0.01	+0.03	0.01	+0.05	—0.01	+0.17	0.01	—0.02	+0.04	+0.22	0.02
7.5	+0.02	—0.02	+0.03	0.01	+0.06	—0.01	+0.25	0.01	+0.01	+0.01	+0.08	0.02
9.0	+0.01	—0.01	+0.03	0.01	+0.04	—0.01	+0.15	0.01	+0.03	+0.04	+0.16	0.02

Table IX shows the effect of removing the cotyledons at *c*₁ and *c*₂ in Fig. 2 when the shoot is supplied with air and the roots with cylinder nitrogen. Again, with the intact seedling there is an evolution of oxygen and absorption of nitrogen which both cease abruptly when the cotyledons are removed, the effect being indistinguishable from that of decapitation.

TABLE IX

Experiment showing the Effect on the Gaseous Exchange of the Root of removing the Cotyledons when the Root is supplied with Nitrogen and the Shoot is exposed to Air. (The first three sets of values are obtained with the intact plant, the last three with the cotyledons removed. Values in columns 1, 2, and 3 represent gas (ml.) absorbed (—) or evolved (+) per hour by the roots of two seedlings; and those in column 4 water (ml.) transpired by the two seedlings per hour)

Time in hours.	1 N ₂	2 O ₂	3 CO ₂	4 H ₂ O
1.5	—0.69	+0.67	+0.39	0.18
3.0	—0.29	+0.27	+0.36	0.20
4.5	—0.32	+0.30	+0.37	0.20
	Cotyledons removed			
6.0	+0.03	—0.01	+0.31	0.08
7.5	—0.00	+0.02	+0.42	0.09
9.0	—0.01	—0.03	+0.45	0.08

DISCUSSION OF RESULTS

Certain of the experiments described above are similar in principle to others designed by earlier workers who have recorded similar results. Cerighelli

(1921) enclosed the roots of intact plants of a number of species in glass cylinders containing pumice. The cylinders were closed by a complicated seal, traversed by the lower part of the stem, and consisting of successive layers of pitch, paraffin wax, gelatin, and mercury. The absolute quantities of oxygen absorbed and carbon dioxide evolved were determined with the intact plant and again after excising the shoot above the seal. The effect of the mutilation was that of decreasing the rate of oxygen uptake and of increasing that of carbon dioxide production, and was thus similar to that shown by the comparable data of Table III. Cerighelli does not discuss the significance of the reduction in the rate of oxygen uptake, but suggests that the increase in carbon dioxide production is due to the interruption of a stream of this gas carried to the leaves in solution in the transpiration stream. Lowenack (1930) re-examined the effect of removing the shoot on the exchange of the root with respect to the respiratory gases, but was unable to confirm the results of Cerighelli. The techniques of Lowenack, however, are open to serious objection since they involved measuring the change in the amount of oxygen dissolved in water in which roots had been immersed without any apparent agitation of the liquid. In these circumstances it is highly probable that the supply of oxygen to the roots was severely restricted.

Cannon (1925, 1925a, 1932) recorded some observations which are consistent with the results of Tables VIII and IX. This worker supplied nitrogen to the roots of intact plants of a number of species in a system in which the atmosphere external to the roots was separated from the air to which the shoot was exposed except through the tissues of the stem, and observed an increase in the oxygen content of the atmosphere around the roots. Moreover, it was found with *Salix* cuttings that the amount of oxygen leaking into the root atmosphere was greater after the expansion of the leaves than it was before it. Cannon suggested that oxygen is translocated from the leaves to the roots through the xylem.

The experiments detailed in the present paper show that when air is supplied to the root and the shoot and the plant is intact, nitrogen is expelled from the root (Tables I, II, III, IV, V, and VI), the origin of which is indicated by the effect of isolation (Table II), decapitation (Table III), and removal of the cotyledons (Table IV). In each case the treatment occasions an immediate cessation of the evolution of nitrogen from the root, which indicates that any operation which separates the cotyledons from the roots interrupts a downward stream of nitrogen which starts in the cotyledons and terminates in the root.

The various treatments that stop the outflow of nitrogen from the root when root and shoot are in air also have another effect, that of an immediate decrease in the uptake of oxygen. Moreover, the decrease continues progressively for some time after decapitation, isolation of the root, or removal of the cotyledons. This aspect of the position is emphasized by the data of Table X, in the experiments of which seedlings are decapitated in the usual way by cutting across the hypocotyl at *a* in Fig. 2 and the exchange of the

root determined over the subsequent $7\frac{1}{2}$ hours, and it is evident that the progressive decrease in the rate of oxygen uptake continues over the whole of this period. The decrease in oxygen uptake is accompanied by an immediate increase in carbon dioxide production (in most instances) followed by a progressive decrease in the rate of this process which again, as shown by the data of Table X, lasts for at least $7\frac{1}{2}$ hours. Further, as a result of separating the root from the cotyledons (by whatever operation) there is an immediate increase in the value of the exchange quotient, which, however, then continues to increase progressively for at least $7\frac{1}{2}$ hours; which indicates that the decrease in the rate of oxygen uptake is always relatively greater than the decrease in the rate of carbon dioxide production.

TABLE X

Two Experiments showing Change in Root Exchange Rates with Time after the Shoot is removed by cutting across the Hypocotyl at Level a in Fig. 2. Air supplied to Root and to Hypocotyl Stump. (Values in columns 1, 2, and 3 represent gas (ml.) absorbed (—) or evolved (+) by two roots per hour; those in columns 4 the corresponding exchange quotients; and those in columns 5 water (ml.) transpired by two seedlings per hour)

Hours.	Expt. 1.					Expt. 2.				
	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O	1 N ₂	2 O ₂	3 CO ₂	4 E.Q.	5 H ₂ O
1.5	+0.01	-0.49	+0.51	1.03	0.02	+0.03	-0.65	+0.64	0.98	0.03
3.0	+0.02	-0.37	+0.44	1.18	0.03	+0.05	-0.51	+0.53	1.04	0.03
4.5	+0.02	-0.37	+0.51	1.34	0.02	-0.00	-0.37	+0.42	1.12	0.02
6.0	+0.01	-0.37	+0.46	1.24	0.04	+0.03	-0.45	+0.52	1.16	0.01
7.5	+0.04	-0.25	+0.32	1.28	0.03	-0.01	-0.22	+0.45	2.14	0.02

Now when the seedling is decapitated or the cotyledons are removed either a hypocotyl stump or an entire hypocotyl extends from the root chamber into the atmosphere external to it, and the decrease in oxygen uptake from the local environment might be due to the induction, as a result of decapitation, of a downward stream of oxygen from the atmosphere to which the hypocotyl is exposed. The data of Tables VII, VIII, and IX, however, show that no downward movement occurs after decapitation and removal of the cotyledons. Further, when the root is isolated (Table II) and there is no connexion with the external atmosphere the general characteristics of the position are the same as when the root is attached to a hypocotyl stump. Alternatively, the immediate decrease in oxygen uptake might be due to the interruption of an upward stream of oxygen. In the intact plant if some of the oxygen that is absorbed by the root escapes upwards into the shoot, then the removal of the shoot might be followed by a reduced uptake of oxygen by the root. The immediate increase in carbon dioxide production is compatible with this interpretation, since if a gas stream flows from the root to the shoot it may carry with it some carbon dioxide, and the interruption of the stream would in that case lead to the diversion outwards of the amount that previously moved upwards. But the immediate effect of removing the shoot or the

cotyledons is probably only the initial stage in a protracted process that occupies at least $7\frac{1}{2}$ hours; whereas the interruption of an upward stream of gas should lead to the immediate establishment of different but stable exchange rates. Clearly the decreases in the rates of oxygen uptake by the root must be attributed to decreasing rates of consumption. The increase in the value of the exchange quotient, however, indicates that the decrease in consumption cannot be due to the development of starvation conditions occasioned by the interruption of a stream of nutrients from the cotyledons. The data of Table VI confirm this conclusion, for in the experiments from which these were obtained a progressively decreasing oxygen uptake and carbon dioxide production with increasing exchange quotients are induced by splitting the hypocotyl longitudinally. In these circumstances vascular continuity between the cotyledons and the root is maintained, and the supply of nutrients to the root therefore continues unimpeded. The increasing value of the quotients with progressively decreasing oxygen uptake might be expected, however, if conditions in the root for respiration become progressively anaerobic, these conditions being induced by an increasing resistance to the inward diffusion of oxygen. But, while it is probable that the primary factor involved in the decreasing oxygen uptake is increased resistance to the lateral diffusion of this gas, it must be emphasized that the data available do not exclude the possibility that some oxygen is carried up from the root in an upward gaseous stream, and although the increase in the rate of carbon dioxide output is not incompatible with the development of anaerobic conditions, nevertheless it may be one of the effects of the interruption of a gas stream flowing to the shoot. The origin of the increasing resistance to the inward diffusion of oxygen is discussed below.

Strong confirmatory evidence for the occurrence of a flow of gases from the cotyledons to the root is provided by the data of Tables VII, VIII, and IX, which show that hydrogen and oxygen flow to the root and are evolved from it when these gases are supplied to the shoot but not to the root. Moreover, the results of these tables agree with those of Tables I, II, III, IV, and V, which indicate that nitrogen flows from the shoot to the root when both are supplied with air, in showing that the downward flow cannot be due to processes of simple diffusion. When shoot and root are in air the partial pressure of nitrogen at the two extremities of the flow-path are the same, and therefore an overall gradient along which diffusion could occur does not exist. In the experiments of Tables VII, VIII, and IX concentration differences are provided and with the intact seedling the flow occurs in the direction of decreasing concentration, but it is evident in these experiments from the results of decapitation that the flow is not determined in all sections of the flow-path by appropriate partial pressure gradients. When the seedling is decapitated the hypocotyl is severed in a region exposed to the atmosphere surrounding the shoot, and as a result of the exposure of the cavity of the hypocotyl the gases surrounding the shoot become immediately accessible to the upper extremity of the root. Nevertheless, when the shoot only is provided

with hydrogen or oxygen and these gases flow to the root, on decapitation the flow ceases immediately, although this operation, while shortening the flow-path, does not affect the concentration difference between the extremities of that path. Further, since the cessation in the outflow of hydrogen or oxygen from the root is abrupt, it indicates that the interruption of the downward flow of these gases is not due to a change in the conditions for diffusion flow in the root. Although decapitation when shoot and root are in air is probably accompanied by increased resistance to transverse diffusion of oxygen, this change is gradual, and not abrupt, as is the change indicated by the cessation in the outward diffusion of nitrogen, hydrogen, and oxygen in the experiments of Tables II, III, IV, VII, VIII, and IX.

But although diffusion—whether simple or activated—over the whole length of the flow-path is excluded, diffusion may still occur over certain sections of it. Since the downward flow starts from the cotyledons, and since the tissues of these are in communication with the atmosphere through the stomata, the gases must be brought to the seat of induction of the downward flow by simple diffusion. Also, since all evolution of nitrogen, hydrogen, and in appropriate circumstances oxygen, ceases when the plant is decapitated, it is evident that active translocation of gases does not occur at any point in the isolated root, which suggests that the exchange between the root tissues and the immediate environment again depends on simple diffusion. Thus the evidence indicates that it is only within the course of the longitudinal flow that non-diffusional processes are involved.

The interpretation in these terms of the flow of nitrogen from the cotyledons to the root when root and shoot are exposed to air carries certain important implications. If the outflow of nitrogen from the root is due to a simple diffusion process, then the partial pressure of nitrogen in the root must be higher than it is in air, and nitrogen must flow to the root from the cotyledons against the incident partial-pressure gradient. The position indicates that the downward flow must be independent of the concentration of gas at the point of delivery and influenced mainly by the partial pressure at the point of absorption.

The results of experiments in which hydrogen and oxygen are presented only to the shoot suggest that all gases presented to the shoot are absorbed by the cotyledons and translocated to the root, in which case it is highly probable that when root and shoot are exposed to air the downward translocation of nitrogen is accompanied by a downward translocation of oxygen. The flow of oxygen is not accompanied by an outward diffusion from the root as with nitrogen, since the consumption of oxygen in respiration reduces the partial pressure of this gas in the tissues of the root to a lower level than it is in air.

The results of experiments in which air is supplied to the shoot and nitrogen to the root indicate that, when both parts of the plant are exposed to air, in addition to a downward flow of nitrogen and oxygen a simultaneous upward flow at least of nitrogen may occur. The results of Tables VIII and IX show that when the shoot is exposed to air and the root to nitrogen the evolution of

oxygen is accompanied by an absorption of nitrogen by the root. On decapitation or removal of the cotyledons both processes cease abruptly, and it is therefore probable that these operations, while interrupting a downward stream of oxygen, also interrupt an upward stream of nitrogen. Moreover, the same considerations that indicate that the downward flow is not determined by diffusion also indicate that the upward is not so determined either. Thus an upward translocation movement of nitrogen may also be involved when air is supplied to both the root and the shoot. No direct evidence is available in any connexion for an upward translocation of oxygen from the root, but if the mechanism involved carries upwards any gases presented to it in the root, then oxygen may stream upward with the nitrogen. But since the amount translocated must depend on the partial pressure of oxygen in the root tissues, the amount that flows upwards must be small, and certainly not comparable in volume to the upward translocation of nitrogen indicated by the data of Tables VIII and IX.

The data presented above, while they show that in normal circumstances movements of gases occur from the shoot to the root and probably also in the reverse direction, also show that this movement cannot be due to simple diffusion. Some form of active translocation must be involved which requires the presence of the cotyledons and of the roots. It is a significant feature of the position that when the root and the shoot are exposed to air the evolution of nitrogen into the root chamber ceases not only when the cotyledons are removed but also when the root is reduced to a short stump (Table V). Moreover, it is evident from the data of Table VI that at least the downward flow probably occurs in solution. In the experiments of Table VI the longitudinal splitting of the hypocotyl exposes the intercellular space system to the atmosphere, and if the translocation occurs by some process such as mass flow along these tracts, then the interruption of the intercellular continuity between the cotyledons and the root should lead to a cessation or acceleration of the flow. The data of Table VI show that the downward stream is not affected and it is therefore probable that the stream of nitrogen, together with the oxygen that accompanies it, flows along either the vascular or the parenchymatous tissue in solution. At the same time the continuation of the evolution of nitrogen also indicates that the partial pressure of this gas in the tissues of the root remains more or less constant, and since in the intact seedling this is partly determined by the upward translocation of this gas, the position indicates that the upward stream is also unimpeded, and therefore also occurs in solution through the parenchyma or vascular tissue of the hypocotyl.

The splitting of the hypocotyl, however, while it has little or no effect on the evolution of nitrogen from the root, has a profound effect on the exchange of the respiratory gases. As indicated above, in this respect the effect of splitting is similar to that of total decapitation. With both there is a progressive decrease in the rate of oxygen uptake accompanied by a progressive increase in the value of the exchange quotient. The considerations that show

that with decapitation this effect is not due primarily to the interruption of an upward gaseous stream are relevant also in this connexion and indicate the same conclusion, namely, that the effect must be attributed to an increasing resistance to the inward diffusion of oxygen into the root. Although it must be emphasized that the general effect of decapitation does not preclude the possibility that before the operation some oxygen escapes from the root into the shoot, so the general effect of splitting does not preclude the complementary possibility that oxygen still continues to escape into the shoot after the operation.

The origin of the increase in resistance to the absorption of oxygen cannot be determined with any confidence from the data available, but the following purely speculative suggestion may be considered as a basis for a further experimental treatment of the problem. If the resistance to mass flow through the intercellular space system is high, and if the partial pressure of gas in the intercellular spaces of the root does not affect the rate of downward translocation, then the discharge of gases into the intercellular spaces may lead to the development of a total gas pressure higher than that of the atmosphere, and the exposure of the intercellular space system, by splitting of the hypocotyl, may release the accumulated pressure (some slight evidence is available that shows that the pressure in the cavity of the hypocotyl is, in fact, higher than atmospheric). With respect to the inward diffusion of oxygen into the root the reduction in the total gas pressure might be expected to have two effects: an immediate reduction in the volume of the intercellular space system of the root, and thus a reduction of the relative volume of the gaseous path available for diffusion; secondly, the release in pressure might be followed by a slow infiltration of water into the intercellular spaces. That this may occur is indicated by the significant fact that after the splitting of the hypocotyl there is a slight increase in the rate of transpiration (Table VII). A slow infiltration of water into the intercellular spaces would account for the progressive increase in resistance to lateral diffusion of oxygen.

It may be pointed out with regard to Table VI that the interpretation for the change in the position with respect to the respiratory gases is not necessarily incompatible with the interpretation given for the apparent stability in the rate of nitrogen evolution. Although the reduction in the total gas pressure might be expected to reduce the partial pressure of nitrogen, this effect is no doubt compensated by the reduced uptake of oxygen.

If the increase in resistance to the diffusion of oxygen is due to the reduction in total gas pressure in the root, this effect should also follow when the downward stream of gas is interrupted without disturbing the intercellular space system. This situation arises when the cotyledons only are removed and it is significant that in this instance, too, the reduction in oxygen absorption is again observed (Table IV). When the seedling is decapitated, of course, not only is the intercellular space system exposed, but the downward stream of gas is also interrupted.

The present series of observations provide no indication as to the mechanism

by which the flow in solution is maintained. It may, however, be pointed out that the cotyledons and the root which are both necessary for the maintenance of the flow have this characteristic in common that they both expose a large vascular surface, and it is therefore possible that it is the vascular tissue that is primarily involved in gaseous translocation. Cerighelli (1921) has suggested that carbon dioxide is carried upwards in the transpiration stream and Cannon (1932) that oxygen is carried downwards in the xylem. But if the xylem is involved then some relation with transpiration might be expected. The data on transpiration obtained in each series of experiments have been given in the tables of the last section since they are of some significance in this connexion. It is evident that there are neither direct nor inverse relations between the rates of transpiration and the rates of oxygen uptake and carbon dioxide production. Again, it is evident that the volume of the transpiration stream is wholly inadequate to provide at least for the upward translocation of nitrogen in solution. Even if the stream is saturated with nitrogen it could only carry about 0.003 ml. per hour, whereas the data of Table VIII show that the amount translocated upwards may be at least a hundred times as great as this amount. If the vascular tissue is involved, however, and the xylem is not, then the possibility arises that the phloem is the primary channel of transport. The very high rate at which the gases are transpired is not incompatible with this suggestion, although there is no direct evidence at present available to support it.

SUMMARY

An apparatus is described for measuring in absolute quantities the gaseous exchange of the root with respect to oxygen, nitrogen, and carbon dioxide, and in circumstances in which different gaseous mixtures are supplied to the root and the shoot.

It is shown that when air is supplied to both the root and the shoot, nitrogen is released from the root; when air is supplied to the root and hydrogen to the shoot, hydrogen is released from the root; and when air is supplied to the shoot and nitrogen only to the root, oxygen is evolved from and nitrogen absorbed by the root. These various processes, however, all cease when the shoot is removed in circumstances in which the atmosphere that initially surrounds the shoot is still accessible to the root. In certain cases the effect on the root exchange has been determined of reducing the size of the root and of removing the cotyledons, and these operations also interrupt the evolution and absorption of nitrogen and the evolution of oxygen.

It is suggested that when both root and shoot are supplied with air, oxygen and nitrogen flow from the cotyledons to the root, and nitrogen with possibly some oxygen in the reverse direction. These processes do not depend on diffusion but on the active translocation of the gases in solution. In this active translocation the cotyledons and the root are mainly concerned.

When the shoot is removed, the rates of oxygen uptake and carbon dioxide production by the root decrease progressively for at least $7\frac{1}{2}$ hours. The

decrease is relatively greater with oxygen uptake. This is interpreted as indicating a progressive development of anaerobic conditions in the root determined by an increasing resistance to the inward diffusion of oxygen.

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Studies in the Physiology of Leaf Growth

IV. The Growth and Behaviour *in vitro* of dicotyledonous Leaves and Leaf Fragments¹

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With six Figures in the Text

INTRODUCTION

IN previous studies in this series it was shown that leaves attached to isolated stem tips of rye embryos were capable of making only very limited growth on any of the media tested (de Ropp, 1945). Such leaf growth as was observed appeared to result rather from the enlargement and differentiation of cells already present than from any increase in cell numbers such as occurred when the leaves were left attached to the plant (de Ropp, 1946). It was also shown that, when such isolated stem tips regenerated roots, rapid elongation of the internodes followed, accompanied by increasingly large growth of the successive leaves (de Ropp, 1946a). Other work bearing on this subject was reviewed in a previous paper (de Ropp, 1945).

The object of the present study was to discover the extent to which the above findings apply to leaves of dicotyledonous plants and to throw some light on the general problem of growth and differentiation of such leaves.

MATERIALS AND METHODS

It was obligatory for the purpose of these experiments that some plant should be used from which leaf tissues at several stages of development could be obtained in a sterile condition. A satisfactory source of such material was found to be the 'head' of the winter type of cabbage which contains within it a series of leaves of increasing immaturity folded tightly within one another. Cabbages of this type were purchased and the outer leaves removed. Discs of tissue were cut from the inner leaves, using a 1-cm. diameter cork borer. In some experiments small entire leaves were cultured. These were excised flush with the stem from the region of the growing-point. The initial weights of discs and whole leaves were obtained by transferring them to freshly weighed tubes of agar, the increase in weight giving that of the leaf fragment.

The basic medium used in the experiments consisted of a mineral solution made up according to the formula of White (1943) to which there was added 2 per cent. sucrose and 0.8 per cent. agar. The medium was distributed to

¹ This work was done in part with an American Cancer Society grant recommended by the National Research Council Committee on Growth.

150 × 25 mm. Pyrex glass test-tubes in 20-ml. amounts and autoclaved for 15 minutes at 15-lb. pressure. The agar was sloped before it solidified. Leaf discs or entire leaves were placed on the surface of this medium and cultured in incubators maintained at 25° C. Where light was required it was provided by two fluorescent tubes built into the incubator. The leaves were usually cultured for a period of 4 weeks before the amount of growth was measured. Final weights of the leaf discs were obtained by removing them from the tubes, carefully wiping off any attached fragments of agar, and weighing them individually in stoppered weighing-bottles. For dry weights the leaf fragments were dried at 100° C. for 24 hours. In most cases mean values were calculated from at least twenty replicates.

EXPERIMENTAL

1. *The growth of excised discs of cabbage-leaf tissue on media of various compositions*

In these experiments the effects on leaf growth of two components of the basic medium, sugar and mineral salts, were tested separately and together. The effect of adding to this medium a vitamin mixture or a small amount of casein hydrolysate was also tested. The media used were therefore seven in number and had the following compositions: (1) plain agar, (2) agar+mineral salts, (3) agar+sucrose, (4) agar+minerals+sucrose (basic medium), (5) basic medium+vitamin mixture, (6) basic medium+casein hydrolysate, (7) basic medium+vitamin mixture+casein hydrolysate. The vitamin solution had the following composition: thiamin, niacin, riboflavin, pyridoxine, pantothenic acid, para-amino-benzoic acid (100 γ per ml.), biotin (0.05 γ per ml.). One ml. per litre of this solution was added to the culture medium before autoclaving. The casein hydrolysate was neutralized with CaCO_3 , autoclaved separately, and added to the medium to give a concentration of 1 mg. per 20 c.c.

The discs of leaf tissue used in this experiment were removed from mature leaves towards the outside of the cabbage. They were laid flat on the agar slopes and incubated for 4 weeks, one set being cultured in continuous light, the other in continuous darkness. From the leaves which provided these discs a second sample of discs was removed, from which the mean initial fresh and dry weights of the samples were calculated. At the end of the 4-week growing period the discs were removed and freed of adherent agar, their individual fresh weights and collective dry weights obtained, and changes in structure and external appearance noted. The mean values of these fresh and dry weights are shown in Table I.

It appears that the discs cultured in light all gained significantly both in fresh and in dry weight during the 4-week period of incubation. This increase in the leaf discs grown on plain agar must have been due entirely to photosynthetic activity. Addition of sugar, minerals, and accessory factors brought about further weight increases, but in view of the large standard of errors of these means it is not possible to take these increases as being significant.

TABLE I

Growth of Isolated Leaf Discs on Media of Various Compositions after 4 Weeks' Incubation at 25° C. (Means of 20 estimations)

Medium.	Light.		Darkness.	
	Fresh wt. (mg.).	Dry wt. (mg.).	Fresh wt. (mg.).	Dry wt. (mg.).
A	114 ± 11	16	66 ± 6	8
AM	111 ± 12	17	63 ± 5	9
AS	127 ± 14	17	127 ± 11	17
AMS	127 ± 13	16	117 ± 11	16
AMSV	132 ± 12	19	132 ± 12	19
AMSC	128 ± 13	17	128 ± 11	17
AMSVC	131 ± 12	18	141 ± 14	18
Initial weights	66 ± 5	9	66 ± 5	9

A agar, M mineral solution, S 2 % sucrose, V = vitamin solution, C = casein hydrolysate

The discs grown in darkness did not show any increase in weight after the 4-week incubation period unless sugar was added to the medium. When sugar was added the amount of growth made was comparable to that of isolated fragments in light. This growth was not increased significantly by the addition of casein hydrolysate or vitamin mixture. As long as sugar was added to these leaf cultures they grew just as much in darkness as in light.

2. Growth of discs of cabbage-leaf tissue from leaves of different ages

The 'head' of cabbage consists of a condensed stem, the leaves of which are tightly folded about one another. These leaves become progressively younger as one approaches the growing-point. The relationship of age of leaf to its size (as measured by fresh weight) is shown in Fig. 1. The oldest, most exterior leaves, which were green in colour, were of smaller size than the outer layers of interior, colourless leaves. These became progressively smaller until primordia near the growing-point were obtained consisting only of ridges of undifferentiated cells. Information regarding the cell size of leaves of different sizes was obtained by clearing fragments of leaves in glycerin and making drawings both of the epidermal cells and of cells of the immediately underlying mesophyll. The average sizes of these cells were estimated by cutting out and weighing the paper on which they were drawn and dividing the weight by the number of cells. In Table II these relative cell sizes (expressed in terms of mg. of the paper) are shown, and the appearance of the cells before and after culture is shown in Fig. 2.

That the weight increases of isolated leaf fragments were due to cellular enlargement rather than to an increase in cell numbers is evident when the cell sizes in Table II are compared with the weight increases given in Table III. In this last table the final weights of discs from leaves of different ages are recorded after they had been cultured for 4 weeks both in light and

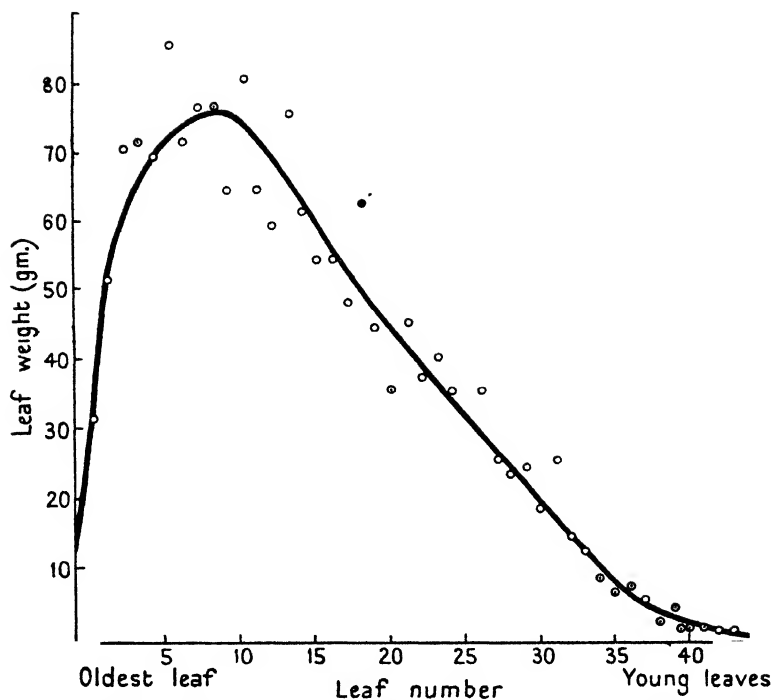


FIG. 1. Relation of weights of cabbage leaves to their ages, i.e. positions on stem.

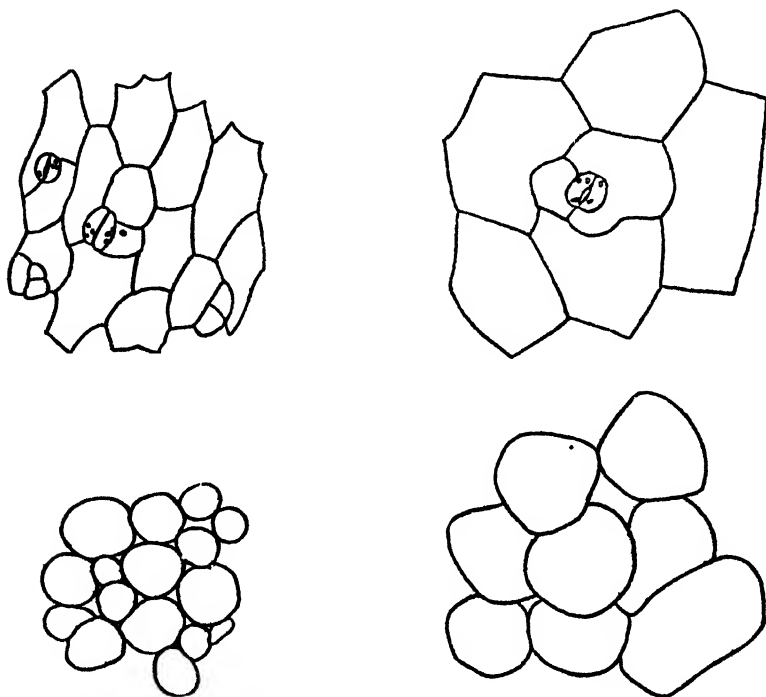


FIG. 2. Enlargement of cells of leaf tissue after 4 weeks' culture *in vitro*. Top, epidermal cells; bottom, mesophyll cells; original size of cells, left; size of cells after culture, right. ($\times 220$.)

in darkness on sucrose-mineral agar containing casein hydrolysate and vitamin mixture.

TABLE II
Average Relative Sizes of Cells of Cabbage Leaves of Different Ages before and after 4 Weeks' Culture in vitro

Leaf weight (gm.).	Epidermal cell size.		Mesophyll cell size.	
	Before culture.	After culture.	Before culture.	After culture.
1	4.5	12.2	10.2	20.0
10	9.4	21.6	10.4	21.2
56	25.2	58.0	24.2	34.4

TABLE III
Growth of Leaf Discs excised from Leaves of Different Ages after 4 Weeks' Culture in Light and Darkness (Means of 20 estimations)

Leaf weight (gm.).	Light				Darkness.			
	Initial weight (gm.).		Final weight (gm.).		Initial weight (gm.).		Final weight (gm.).	
	Fresh.	Dry.	Fresh.	Dry.	Fresh.	Dry.	Fresh.	Dry.
1-10	73 ± 5	9	177 ± 16	23	71 ± 3	8	183 ± 17	27
11-20	71 ± 3	10	165 ± 17	21	72 ± 5	9	181 ± 18	28
21-30	65 ± 4	9	118 ± 10	16	65 ± 4	9	132 ± 12	19
31-40	70 ± 5	8	120 ± 12	18	69 ± 5	8	131 ± 13	17
41-50	69 ± 4	9	125 ± 13	17	63 ± 3	8	119 ± 10	16
Over 50	62 ± 4	8	116 ± 11	15	68 ± 4	9	125 ± 11	18

It appears that the amount of growth made by discs taken from leaves weighing less than 20 gm. was significantly greater than that made by discs from older leaves. Although it was evident that the growth observed resulted mainly from the enlargement of existing cells, fairly massive outgrowths of callus tissue often occurred at the cut edges of the discs (see Fig. 5). The appearance of these discs after 4 weeks' growth in light or darkness is shown in Fig. 3.

3. Effect of roots on the growth of leaf discs and whole leaves cultured in vitro

The regeneration of roots occurred spontaneously in as many as half of the leaf discs cultured in the foregoing experiments. Roots were also often regenerated by entire leaves cultured *in vitro* (see Fig. 4). The roots were always regenerated from veins. In order to determine whether this production of roots resulted in any increase in the amount of growth made by the discs of leaf tissue, the weights of rooted discs were collected and compared with those of unrooted discs grown under similar circumstances. The mean fresh weight of rooted discs was 128 ± 13 mg., that of the rootless fragments was 125 ± 12 mg. This difference is not significant and shows that leaf tissue was not stimulated to grow by being attached direct to a root system. It was found, however, that when entire stem tips were cultured, the young leaves attached to such stems would only grow when roots were regenerated by the stem. This confirms a previous finding (de Ropp, 1946a) which showed that

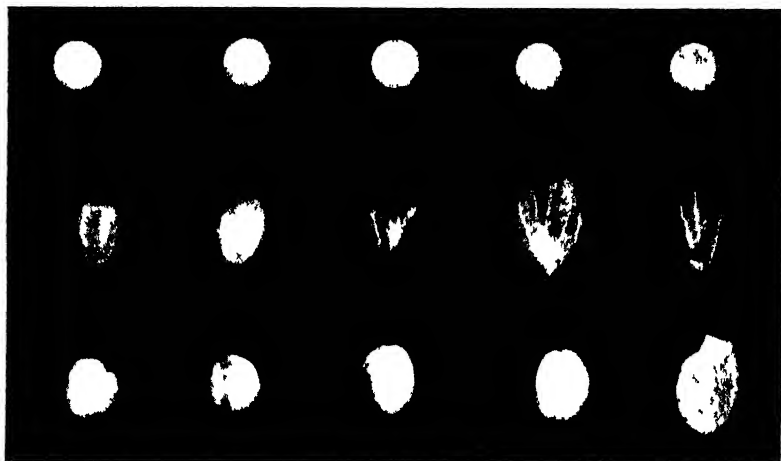


FIG. 3. Appearance of discs of leaf tissue after 4 weeks' culture on sucrose mineral agar in light and darkness. Upper row, original size of discs; middle row, after 4 weeks' culture in light; lower row, after 4 weeks' culture in darkness.

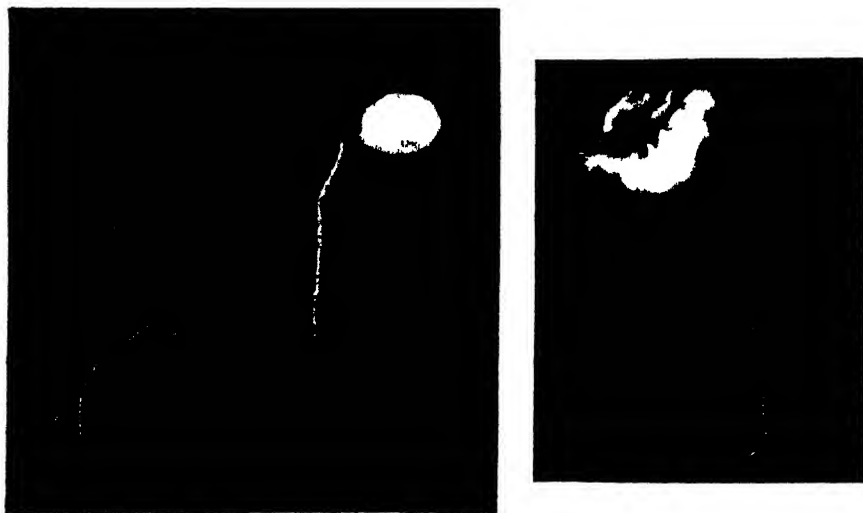


FIG. 4. Left, root production by isolated leaf disc, right, by isolated whole leaf
isolated embryonic stem tips of rye would only develop after the stem had regenerated roots. The complete system, root-stem-leaf, seems to be necessary for leaves to respond to the action of roots. The root alone does not appear to stimulate leaf growth.

4. *Effect of indole acetic acid on the growth of isolated cabbage-leaf tissue*

In order to test the effect of this substance on cabbage-leaf tissue cultures were set up on sucrose-mineral agar containing 100 and 1 mg. per litre of

indole acetic acid respectively. The hormone was sterilized by filtration and added to the agar as it cooled. Discs of leaf tissue from fully mature leaves and from young leaves of less than 10 gm. weight were placed on this agar. After 4 weeks' culture in both light and darkness the discs were removed and their fresh and dry weights determined. Mean values of these weights are shown in Table IV.

TABLE IV

Growth of Cabbage-leaf Tissue on Sucrose-mineral Agar with two Concentrations of Indole Acetic Acid after 4 Weeks' Culture in Light and Darkness (Means of 20 estimations)

				Light.		Darkness.	
				Fresh wt. (mg.).	Dry wt. (mg.).	Fresh wt. (mg.).	Dry wt. (mg.).
Mature tissue	100 mg. per l.	.	.	93 ± 10	12	85 ± 9	12
	1 " " "	.	.	125 ± 11	18	127 ± 11	18
	No hormone	.	.	121 ± 11	17	118 ± 12	17
Young tissue	100 mg. per l.	.	.	110 ± 11	15	102 ± 10	14
	1 " " "	.	.	171 ± 16	21	182 ± 17	26
	No hormone	.	.	176 ± 15	22	179 ± 18	28
Initial weights				61 ± 4	8	69 ± 5	9

Evidently the high concentration of indole acetic acid significantly reduced the amount of growth of the leaf discs. The lower concentration did not have any effect on the growth of this tissue. The outgrowth of callus which formed around the cut surface of these discs was completely inhibited by the high concentration of the hormone (Fig. 5). Mature and young leaf tissue reacted to the hormone in a similar manner, and there were no indications that indole acetic acid had the power to evoke root formation in these isolated discs, although it had already been shown that the leaf tissue was capable of generating roots spontaneously.

When very young leaves were removed intact from the stem and cultured with their bases resting on agar containing 100 mg. per litre of indole acetic acid, a different reaction was observed (Fig. 6). Such leaves became swollen at the base, the tissues of which proliferated to give rise to a mass of brown, disorganized tissue from which numbers of thin, weak roots projected. The proliferation was confined to the tissue of the midrib and did not take place at all in the lamina. No proliferation was observed in leaves which had been cultured in the presence of the lower concentration of indole acetic acid. On transfer to hormone-free agar, these tissue masses continued to give rise to roots. This behaviour

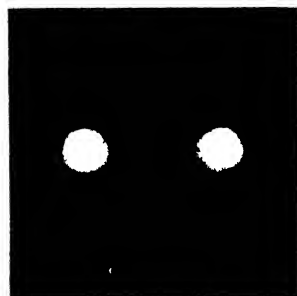


FIG. 5. Growth of discs of cabbage leaf after 4 days on agar containing indole acetic acid. Left, 100 mg. per litre indole acetic acid; growth of callus suppressed. Right, 1 mg. per litre indole acetic acid; growth of callus normal.

paralleled the reactions of normal sunflower tissue to high concentrations of indole acetic acid. It is evident from these results that the very young tissue of the midrib reacts quite differently to indole acetic acid from the older lamina tissue. It also appears that cabbage tissue responds to very high concentrations of indole acetic acid (100 mg. per l.) in contrast to such tissue as



FIG. 6. Effect of indole acetic acid on growth of entire cabbage leaves cultured for 4 weeks in darkness. Left, leaf on agar containing 100 mg. per litre indole acetic acid. Right, leaf on agar containing 1 mg. per litre indole acetic acid.

sunflower or periwinkle which respond to as little as 0.001 mg. per l. (de Ropp, 1947).

CONCLUSIONS AND SUMMARY

Cabbage-leaf tissue was shown by these experiments to be capable of making a limited amount of growth when cultured *in vitro* on agar containing 2 per cent. sucrose and mineral salts. The addition to this medium of casein hydrolysate and of thiamin, pyridoxine, niacin, riboflavin, pantothenic acid, para-amino-benzoic acid, and biotin did not bring about a significant increase in the amount of growth. When cultured in light the weight of leaf fragments increased even on plain agar, showing that they had a capacity for photosynthesis.

Growth of isolated leaf discs appeared to result from an increase in cell size rather than cell number, though the production of callus from the cut edges of the leaf was observed in many cases. The amount of growth made by fragments of young leaves was significantly higher than that made by mature leaf tissue.

Discs of isolated leaf tissue proved capable of regenerating roots from the cut surface of veins. These roots grew freely but did not bring about any increase in growth of the leaf fragment itself. When roots were regenerated by an isolated growing-point, the leaves began to expand, suggesting that the

system, root-stem-leaf, is required before leaves will grow under the stimulus of the roots.

Isolated cabbage-leaf tissue proved very insensitive to indole acetic acid, showing no response to a concentration of 1 mg. per litre and inhibition of growth in a concentration of 100 mg. per litre. Very young cabbage leaves excised whole from the stem showed no response to 1 mg. per litre of this substance, but in the presence of 100 mg. per l. the leaf bases became swollen and proliferated, giving rise to a mass of roots. The tissue of the lamina did not respond to the hormone.

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NOTE

Diurnal changes in the area of Cacao leaves.—Since Thoday's (Proc. Roy. Soc., B, lxxxii, 1-55, 1909) exhaustive investigation of the sources of error involved in the half-leaf method of Sachs (Arb. Bot. Inst. Würzburg, iii, 1-33, 1884) it has been generally accepted that changes in leaves expressed on an area basis cannot be directly converted to other terms on account of the diurnal variations in area of leaves. Thoday found that the area of *Helianthus annuus* leaves frequently decreased by 5 per cent. between dawn and midday or early afternoon, the average decrease for four leaves measured on six days being 3.7 per cent. The decreases appeared to be the result principally of direct insolation. Other plant species gave similar results; even the firm leaves of cherry laurel decreased in area by 1.2 per cent. without showing any signs of decreased turgidity. Changes of the order of 5 per cent. are likely to invalidate completely any measurements of leaf dry-weight change based on dry-weight determinations for fixed areas of leaf tissue, and Thoday accordingly proposed modifications in Sachs's technique to take area changes into account. These modifications, however, considerably reduce the convenience of the method, and are to be avoided, if possible without seriously impairing its accuracy.

It seemed reasonable to suppose that the area changes in leaves of temperate plants under full insolation might not occur in cacao, which in the Eastern Province of the Gold Coast forms part of the lower canopy of a tropical forest, where conditions tend to be more uniform and leaf insolation only fleeting. Accordingly a series of observations on area change in cacao leaves was performed. These observations began in August, in the middle of an exceptionally severe short dry season, during which the rainfall over a period of 9 weeks amounted to only 88 mm., and the average hours of sunshine per day were 2.58. The shorter wet season began at the end of September, the rainfall during the following two months being 607 mm., and the average hours of sunshine 5.36 per day. Observations extended into December, by which time the main dry season had begun. The experimental material included plants of all ages from seedlings less than 1 year old to mature trees of 20 years or more, all under natural shade provided by plantains or forest trees; leaves of both fan and chupon shoots were measured.

Two methods of area measurement were used. The first consisted of direct measurement (with a millimetre rule) of the maximum length and breadth of the leaf lamina. Thoday's method of marking the leaf surface with ink crosses appeared unnecessary with cacao, the leaves of which are simple and clearly defined in outline. On seven occasions measurements were performed through the 24 hours at 4- to 6-hour intervals on twenty healthy and intact leaves, either from a single tree or from several seedlings growing adjacent one to another. The mean results for each occasion are given in Table I. On none of the occasions were the differences significant, and there is no evidence of any consistent trend with time of day.

The second method of measuring area change was one suggested to the author by the work of B. D. Bolas. Dry methyl violet, mixed with talc dust as a carrier, was rubbed over strips of paper; the leaf was then held against a strip of this prepared paper in a wire frame, and both were sprayed with water from a fine atomizer. The darkening of the methyl violet in contact with water, where it was not protected

TABLE I

*Diurnal Changes in mean Product of Length (cm.) and Breadth (cm.) of Lamina.
(Means of 20 leaves)*

Date.	Approximate hour of observation.						S.E. of Mean Product.
	12.00	16.00	20.00	00.00	06.00	12.00	
30-31/8/46	209.2	209.5	—	208.5	208.7	209.0	0.4
7-8/10/46	324.2	325.7	326.1	325.3	323.6	325.5	1.6
18-19/10/46	280.1	283.2	282.2	281.2	281.3	282.8	1.0
28-29/10/46	274.5	275.4	271.8	271.0	272.2	275.1	2.0
11-12/11/46	390.5	388.7	386.1	390.9	388.6	388.9	1.6
5-6/12/46	199.0	195.9	195.4	196.7	196.0	196.8	1.1
18-19/12/46	292.8	292.5	292.2	291.8	295.3	293.4	1.6

by the leaf, gave a clear outline which could be cut out and retained for area measurement at leisure. Repeated spraying over a period of 20 minutes was not found to cause any appreciable change in leaf area, and the film of water rapidly evaporated from the leaf surface. Since a planimeter was not available, and the treated paper was insufficiently uniform to use a weighing method, the areas of the prints were determined by placing under a sheet of glass ruled in 1-cm. squares ($\frac{1}{2}$ -cm. squares for the smaller leaves), and counting the number of squares covered. As with the measurements recorded in Table I, different trees were selected for each of the occasions over which the observations were made. Twenty separate prints of the leaf chosen were taken at midday, and the process was repeated five or six times during the 24 hours. Table II shows the mean leaf area as determined for the groups of twenty prints.

TABLE II

Diurnal Changes in Area (sq. cm.) of single Leaves

Date.	Approximate hour of observation.							Significant difference.
	12.00	16.00	18.00	20.00	00.00	06.00	12.00	
30-31/8/46	216.53	—	217.62	—	217.99	217.97	217.90	N.S.
2-3/9/46	195.95	—	195.20	—	195.45	194.55	196.75	N.S.
5-6/9/46	361.83	—	368.48	—	366.33	367.73	364.73	2.03***
9-10/9/46	385.40	—	384.60	—	383.25	384.15	381.95	2.04*
12-13/9/46	158.36	—	157.83	—	156.73	157.89	158.86	0.98***
23-24/9/46	173.18	—	172.64	—	173.88	174.75	173.93	N.S.
7-8/10/46	340.25	340.53	—	338.98	340.10	339.23	341.20	N.S.
18-19/10/46	243.40	243.78	—	242.20	242.13	243.43	245.80	0.94***
28-29/10/46	297.33	300.83	—	290.85	298.88	301.78	299.13	1.28***
11-12/11/46	263.05	264.00	—	264.80	262.65	264.30	263.75	N.S.
5-6/12/46	130.59	132.64	—	127.93	131.81	129.85	130.16	0.75***
18-19/12/46	147.48	149.28	—	146.18	147.55	147.23	147.50	1.41**

Results of analysis of variance:

* $P:0.01-0.05$.

** $P:0.001-0.01$.

*** $P < 0.001$.

Significant differences were found on seven of the twelve occasions, though on only two occasions did the extreme range exceed 2 per cent. of the mean. The only discernible consistency in the data is a tendency for the area to increase between noon and 16.00 hours, and thereafter to decrease; this tendency, however, does not reach significance if all six occasions with observations at or about 16.00 hours are considered collectively. No relationship between the observed area differences and

atmospheric humidity could be found. It should be added that, though precautions were taken to prevent spurious differences in the means for different hours arising in the course of measurement of the leaf prints, it was not possible to eliminate the risk of differences in the preparation of the prints, which of course took place under very varied lighting conditions; it is thus not inconceivable that the significant differences observed were in the technique rather than in the leaf area itself.

Observations were also made on changes in the length of leaves of a potted plant not exposed to direct sunlight, an auxanometer trace, with a magnification of four times, being made on a recording drum. The petiole was secured and the leaf held taut in line with the petiole by a rubber band giving a tension of about one pound. Under these conditions, the length increased consistently between 11.00 and 16.00 hours, decreasing gradually thereafter, and regaining a value very close to the original at about sunrise. The diurnal changes in five such experiments are given in Table III, as ranges between maximum and minimum expressed as a percentage of the total free length of the leaf:

TABLE III
Diurnal Changes (per cent.) in Length of a Leaf under Tension

Leaf.	Date of commencement.	Day.					
		1	2	3	4	5	6
A	2/9/46	0·21	0·29	0·49	0·25	0·04	0·25
B	12/9/46	0·23	0·17	0·23	—	—	—
C	24/9/46	0·07	0·07	0·23	0·09	0·29	—
D	30/10/46	0·26	0·21	0·17	0·22	0·09	0·21
E	10/1/47	0·14	0·20	0·27	0·11	—	—

These changes in length are of insufficient magnitude to conflict with the failure to find consistent changes in leaf area, the errors in measurement of which were substantially greater than those in an auxanometer record. Presumably the afternoon increase in length reflects a slight reduction in turgidity consequent upon the daily decrease in atmospheric humidity, the morning mean for the days of observation being 82·0 per cent. and the afternoon mean 61·1 per cent.; the increase in length on a particular day was not, however, correlated with the extent of the diurnal decrease in humidity on that day. The possible region in which turgidity changes might have occurred included, of course, the distal pulvinus and part of the petiole.

Thus, while cacao leaves seem to undergo regular diurnal changes in turgidity, and while the area of individual leaves may not infrequently change during the day by as much as 1 per cent., such changes in area are inconsistent as between different leaves of the same tree, and it has not been possible to detect any diurnal change in the mean area of a random sample of twenty leaves. This suggests that it may well be practicable to use for cacao one of the simpler versions of Sachs's method for determining dry-weight changes, provided material is collected from a considerable number of leaves.

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D. W. GOODALL.

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